BLINDNESS IN CATTLE ASSOCIATED WITH A CONSTRICTION OF THE OPTIC NERVE AND PROBABLY OF NUTRITIONAL ORIGIN [1]

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SIX FIGURES

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During the past 12 years the Dairy Section of the Michigan Agricultural Experiment Station has observed twenty-four cases of blindness in dairy animals used in experiments to study the dietary factors carried by good quality hay. This particular type of blindness is present in calves—apparently developing during the intra-uterine period—whose dams received rations with a poor quality roughage. The blindness may also develop in young growing dairy animals if they receive a ration containing roughage of poor quality.

This particular type of blindness is characterized by a dilated pupil even on exposure to bright sunlight and by the absence of inflammation of the external structure of the eye. In cases following birth, this type of blindness is frequently associated with partial paralysis, especially of the front quarters, weakness, a peculiar position of the head and sometimes spasms, as shown in figure 1. In young growing animals it is associated with stiffness, poor condition, and occasional spasms. There is no known therapy which will restore the eye sight in this type of blindness either in calves blind at birth or in young growing animals. This condition is closely associated with poor reproduction as first reported

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by Hart and co-workers ('11, '17, '20 and '24) when the dam's ration consisted of the oat plant, the wheat plant or a mixture of oat, wheat and corn plants.

In 1926, the blindness in cattle at this station was found to be due to atrophy of the optic nerve at a point where it passed through the optic foramen from the brain. Sectioning the nerve and its bony canal in 1933 showed that the constriction could be due to narrowing of the bony canal.

Cases of blindness in calves and young growing dairy animals have been reported by several investigators. Reed, Huffman and Addington ('28) reported blindness in calves and showed photomicrographs illustrating a degeneration of

Fig. 1 Blind calf of C 200, showing paralysis of front quarters.

the optic nerve. Huffman ('28) also reported blindness in calves when the dams received wheat straw as roughage. The animals considered in the two above mentioned reports are also included in this discussion. Other reports of blindness which may be related to the blindness described in this paper are recorded by Halverson and Sherwood ('30), Converse and Meigs ('32), du Toit, Malan and Groenewald ('34), Hart and co-workers ('24), Datta ('33) and Crocker ('19). Hart and Guilbert ('33) apparently described what is undoubtedly the true vitamin A type of blindness and which is different from the type of blindness reported in this paper. Likewise, Jones, Eckles and Palmer ('26) and Mead and Regan ('31) apparently described the true vitamin A type of blindness. De

Schweinitz ('31) and De Schweinitz and De Long ('34) described a type of blindness due to papilledema (choked disc) which they believe may be an inherited characteristic. It is sometimes difficult to tell from the descriptions in the literature whether the type of blindness is primarily the vitamin A type, characterized by an inflammation of the external eye structures, or is the type of blindness reported in this paper in which there is no evidence of inflammation, but which may or may not be due to vitamin A deficiency.

The purpose of this paper is to report data and describe a type of blindness somewhat different from the true vitamin A ophthalmia.

EXPERIMENTAL

In 1922, work was begun at this station to study the dietary factors carried by good quality hay. This was done by feeding various types of concentrate rations with poor quality roughages and using various supplements. In this report, whenever wheat straw, hay or silage was not fed, the roughage consisted of wood shavings.

All animals were placed on their respective rations at from 3 to 6 months of age. The animals were turned outside during the day to exercise, except during inclement weather, unless otherwise noted. When the animals were kept away from sunlight they were usually turned out at night for exercise. No. 2 yellow corn was used in all rations containing corn. Iodized salt was fed. The cod liver oil used was a good grade of medicinal oil. The viosterol used was 250D. All animals received water, except when considerable milk was fed.

Accurate daily records were kept of food consumption and of any abnormalities which occurred. Blindness in calves was determined following birth by noting the reaction of the pupil of the eyes to bright light from the sun or a flash light. Blindness would first be noticed in young growing dairy animals when they started bumping into objects when they were removed from the stalls to the exercise lot.

Data pertaining to the animals are shown in tables 1 to 5, inclusive.

RESULTS

When the dams of the calves received a ration consisting principally of a grain mixture containing large amounts of corn, corn silage and wheat straw as roughage (table 1), considerable blindness and poor reproduction resulted. This ration should contain fair amounts of vitamin D, but it is low in calcium.

Six definitely blind calves were born from dams on the above ration. Four other calves were dead at delivery and one other calf died before it could be determined whether it was blind. Of the blind calves, the optic nerves of C 35's second calf and C 46's first calf were examined and showed changes. The optic nerves of C 50's first calf were also examined, but they were apparently normal.

Seven strong calves were born. In five of the seven cases of strong calves, their dams, C 46 and C 50, consumed much more silage during the succeeding gestation periods than during the first. In the other two cases the dams' rations were changed from wheat straw to timothy hay and they also consumed much more silage than during the first two gestation periods in which blind calves were produced.

The addition of 100 cc. of cod liver oil per week to the ration of animal 200 during the last 3½ months of gestation did not prevent the development of blindness in the calf. It is possible that the effects upon the fetus had developed before the cod liver oil was added to the ration or that the cod liver oil purchased in 1924 may not have been as rich in the vitamins, as that obtainable today.

When the ration of the dams consisted principally of rolled oats and oat hay, three blind calves were produced, as shown in table 2. This ration was low in calcium and in vitamin D, because the oat hay was cured in the dark, but it probably contained some vitamin A as the hay was green and leafy.

Although O 1's calf was blind, it was able to stand up and was apparently in healthy condition when sold for veal at 39 days of age. O 6's first calf was blind and paralyzed. At autopsy the optic nerves showed atrophy, as shown in

TABLE 1

TABLE 1

Data pertaining to calves whose dams received a ration consisting principally of grain, corn silage and wheat straw

DAM NO.	CALF NO.	DAILY FOOD CONSUMPTION OF DAM DURING GESTATION				DATE OF CALVING	DAYS CARRIED	SEX	WEIGHT	HEALTH AT BIRTH	DISPOSAL
		Grain mix	Corn silage	Wheat straw	Timothy						
		Pounds	*Pounds*	*Pounds*	*Pounds*				*Pounds*		
201	1st	10–12[1]		ad lib.		5–11–24	246	Male	73	Dead at delivery	
205	1st	14[2]				8–25–24	234	Female	38	Blind, paralyzed	Lived 1 day
200[6]	1st	12[1]		ad lib.		9–15–24	244	Female	55	Blind, finally got up	Lived 18 days
C 35	1st	7[3]	7	6–7		8– 8–26	258	Male	68	Blind, strong, stiff neck	Killed 106 days of age
C 35	2nd	12–14[4]	7–12	6–7 + ad lib.		7–14–27	260	Female	73	Blind, weak	Killed, 1 day old
C 35	3rd	8–14[4]	20–35		8–10	7–21–28	280	Male	88	Strong	
C 35	4th	8–14[4]	20–30		8–10	8–21–29	266	Female	79	Strong	Died with navel rupture next day
C 38	1st	7[4]	7	3–6		3–12–26	...	Female	78	Blind, paralyzed	Killed, 11 days of age
C 39	1st	7[5]	7	7		7– 6–26	...	Male	55	Very weak, paralyzed	Died in 19 hours B. coli infection
C 37	1st	7[5]	6– 7	7–8		8–15–26	218	.....	33	Dead at delivery	
C 37	2nd	12[5]	7–11	7 + ad lib.		7–13–27	246	Male	..	Dead at delivery	
C 46	1st	7[3]	7	7		9–26–27	270	Female	70	Blind, weak	Killed 2 days of age
C 46	2nd	12–16[4]	15–25	5–7		10–15–28	276	Female	74	Strong	
C 46	3rd	8–20[4]	30	3–9		10–24–29	282	Male	100	Strong	
C 50	1st	6– 7[3]	4– 7	5–7		2–12–27	263	Male	81	Dead	
C 50	2nd	8–16[4]	12–20	5–7 + ad lib.		4–21–28	282	Male	94	Strong	
C 50	3rd	8–18[4]	20–25	5–9		4–23–29	277	Male	106	Strong	
C 50	4th	8–15[4]	20–30	5–6		8–15–30	292	Male	98	Strong	

[1] 400 pounds yellow corn
50 pounds corn gluten meal
50 pounds cottonseed meal
5 pounds sodium chloride

[2] 100 pounds of [1] plus
5 pounds wheat straw

[3] 300 pounds yellow corn
100 pounds oats
100 pounds cottonseed meal
5 pounds sodium chloride

[4] 300 pounds yellow corn
100 pounds oats
200 pounds linseed oil meal
5 pounds sodium chloride

[5] 100 pounds of [3] plus 3 per cent magnesium phosphate.

[6] Received 100 cc. of cod liver oil one day each week for last 3½ months of gestation period.

TABLE 2

Data pertaining to calves whose dams received a ration consisting principally of rolled oats and oat hay

DAM NO.	CALF NO.	DAILY FOOD CONSUMPTION OF DAM DURING GESTATION				DATE OF CALVING	DAYS CARRIED	SEX	WEIGHT	HEALTH AT BIRTH	DISPOSAL
		Rolled oats	Oat [1] hay	Cod liver oil	Grain mix						
		Pounds	*Pounds*	*cc.*	*Pounds*				*Pounds*		
O 1	1st	6	6			10–24–27	270	Female	63	Blind, able to stand up	Sold, posted 12–12–27
O 6	1st	4– 5	8			5–22–28	252	Female	47	Blind, paralyzed	
O 6	2nd	8–10	8–10	20		4–17–29	278	Male	96	Strong	
O 6	3rd			20	10–12[2]	7–14–30	270	Female	68	Strong	
O 8	1st	5– 7	5– 7			6– 3–29	272	Female	71	Blind, weak	Died 6–7–29
O 8	2nd			20–50	8–15[2]	2–23–31	268	Male	79	Active, normal	

[1] Cured in dark.

[2] 40 pounds linseed oil meal
20 pounds wheat bran
60 pounds yellow corn
60 pounds oats
4 pounds bone meal
4 pounds calcium carbonate
2 pounds sodium chloride

figure 2. O 6 was able to produce a normal calf, however, when the above ration was supplemented with 20 cc. of cod liver oil per day. This animal also produced a normal calf when she received a grain ration supplemented with 20 cc. of cod liver oil per day. O 8 received a ration of rolled oats and oat hay during her first gestation period and gave birth to a blind and weak calf. During the second gestation period O 8 received a grain mixture supplemented with 20 to 50 cc. of cod liver oil per day and gave birth to a normal healthy calf. These two cases furnish some evidence that cod liver

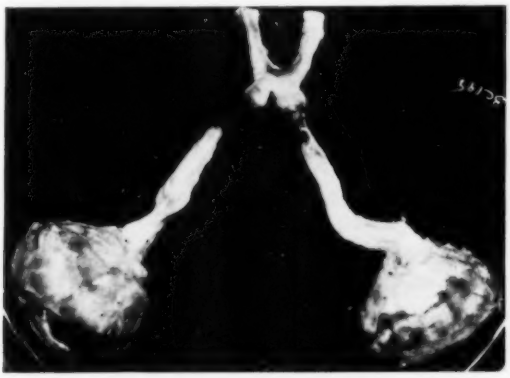

Fig. 2 Constriction of the optic nerve of O 6's first calf.

oil carries the factor or factors necessary to prevent this type of blindness.

When the dam's ration consisted principally of skim milk, corn and oats, as shown in table 3, one blind and one paralyzed calf were produced. C 75 produced a normal calf when she received the above ration supplemented with a 20 to 50 cc. of cod liver oil per day. Her second calf, however, was blind and paralyzed, although she received 50 cc. of cod liver oil per day during the first month of gestation. After the first month of gestation C 75 was kept away from sunlight. At autopsy the optic nerves of C 75's second calf were found to be severely atrophied. C 83 received a ration consisting

TABLE 3

Data pertaining to calves whose dams received a ration consisting principally of skim milk, corn and oats

DAM NO.	CALF NO.	DAILY FOOD CONSUMPTION OF DAM DURING GESTATION					DATE OF CALVING	DAYS CARRIED	SEX	WEIGHT	HEALTH AT BIRTH	DISPOSAL
		Skim milk	Corn and oats	Cod liver oil	Cotton-seed meal	Wheat straw						
		Pounds	*Pounds*	*cc.*	*Pounds*	*Pounds*				*Pounds*		
C 75	1st	16	7–10	20–50			2-28-31	273	Male	82	Strong	
C 75[1]	2nd	30	16–18	50[2]			2- 6-32	243	Female	47	Blind, paralyzed	Killed 2 days of age
C 83	1st		1.5	30	8	2	10–20–31	261	Male	68	Normal	
C 83[3]	2nd	30	5– 8	Ultraviolet 15 min.			5– 1–33		Male	63	Normal	
C 83[4]	3rd	30–35	8–13	15 min.			3–15–34	237	Male	35	Paralyzed	Died in a few hours

[1] Kept from sunlight after first month of gestation.
[2] First month of gestation.
[3] Pasture first 6 weeks of gestation after which did not receive sunlight.
[4] No sunlight.

of cottonseed meal, wheat straw and 30 cc. of cod liver oil per day during her first gestation period. Her first calf was normal. During the first 6 weeks of C 83's second gestation she was on pasture, after which time she was kept away from sunlight, but was irradiated with a quartz mercury lamp for 15 minutes per day and received a ration consisting of skim milk, corn and oats during the rest of the period. Her second calf was also normal. During C 83's third gestation, she received the same ration and treatment as that received after the first 6 weeks of the second gestation period. The third calf, however, was paralyzed at birth and died before it could be determined whether or not it was blind. The optic nerves showed marked atrophy upon sectioning. The blood calcium and inorganic phosphorus values were markedly reduced during the last 6 weeks of gestation.

The ration of the dams shown in table 4 consisted principally of a grain mixture with various supplements. This ration contained considerable vitamin A in the form of yellow corn and corn gluten meal. It also had an optimum calcium to phosphorus ratio, but it was low in vitamin D, except when this was supplied in various forms and amounts, as shown in the table. All of the calves produced were either blind or dead at delivery, with the exception of C 172's calf which was probably killed during delivery. The optic nerves of this calf, however, showed the characteristic degeneration. All of the blind calves in this series showed the characteristic atrophy of the optic nerves. From sections of the nerves of the calves of C 108 and C 118 it was evident that the atrophy could be due to bone pressure. A cross section of the nerve within the bony canal of C 118's calf compared with a similar cross section from a normal calf at birth is shown in figures 3 and 4. Figure 5 shows a longitudinal section of the nerve of C 108's calf.

The sections were made by decalcifying the bone tissue and removing representative sections every 500 μ along the nerve where it passed through the bony canal. It is of interest to observe that the diameter of that portion of the bony canal

TABLE 4

Data pertaining to calves whose dams received a ration consisting principally of a grain mixture

DAM NO.	CALF NO.	DAILY FOOD CONSUMPTION OF DAM DURING GESTATION				DATE OF CALVING	DAYS CARRIED	SEX	WEIGHT	HEALTH AT BIRTH	DISPOSAL
		Grain mix	Cod liver oil	Sunshine and ultra-violet	Magnesium oxide						
		Pounds	*cc.*		*gm.*				*Pounds*		
C 103	1st	10	20	None		12–12–32	247	Male	79	Blind, paralyzed	Lived 3 days
C 106	1st	11		+		5–13–33	279	Female	77	Blind, paralyzed	Killed 1 day of age
C 108	1st	13	20	None		8– 4–33	267	Male	83	Blind, strong	Killed 50 days of age
C 118	1st	12		+		11–19–33	260	Male	69	Blind, paralyzed	Lived 3 days
C 97	1st	11–13		+		10–10–32	254	Male	52	Dead	
(twins)								Male	52	Dead	
C 172	1st	11	5	None	24	4–16–34	263	Female	74	Killed in pulling	
			Viosterol								
C 112	1st	11	20 drops	None		1–26–33	157	.....	..	Dead	

[1] 50 pounds yellow corn
20 pounds oats
20 pounds yellow corn gluten meal
8.5 pounds linseed oil meal
1 pound calcium carbonate
0.5 pound sodium chloride

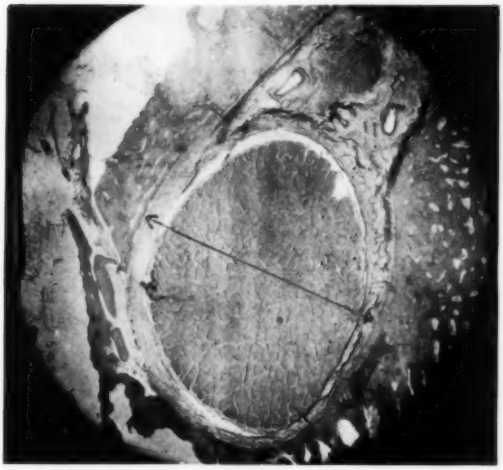

Fig. 3 Cross section of the optic nerve and bony canal of a normal calf at birth. × 16.

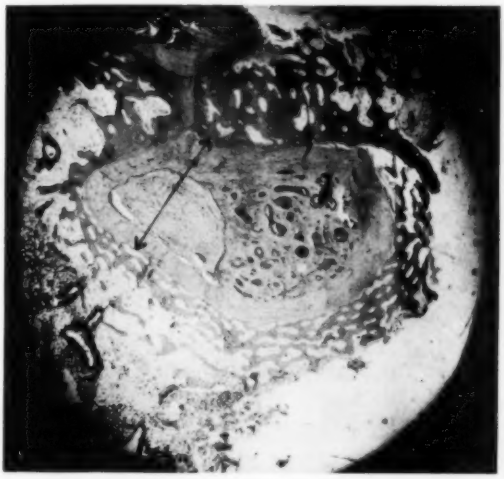

Fig. 4 Cross section of the optic nerve and bony canal of C 118's calf. Note difference in diameter of bony canal compared with the normal. × 16.

through which the nerve passed was only about one-half the normal diameter, as shown in figures 3 and 4. It can also be seen that the nerve is almost completely degenerated, and

Fig. 5 Longitudinal section of the optic nerve and bony canal of C 108's calf. × 16.

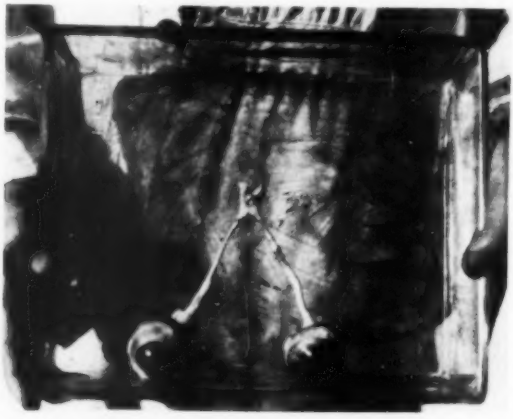

Fig. 6 Constriction of the optic nerve of C 91.

that the connective tissue surrounding the nerve and its accompanying nerves and blood vessels is considerably more dense than the normal.

TABLE 5

Data relative to blind growing dairy animals

ANIMAL NO.	DAILY FOOD CONSUMPTION DURING PERIOD OF DEVELOPMENT OF BLINDNESS					AGE WHEN PLACED ON RATION	AGE WHEN BLINDNESS WAS FIRST NOTICED	DATE WHEN BLINDNESS WAS FIRST NOTICED	DEGREE OF BLINDNESS WHEN FIRST NOTICED
	Grain mix	Potatoes	Timothy hay	Skim milk	Corn and oats				
	Pounds	*Pounds*	*Pounds*	*Pounds*	*Pounds*	*Days*	*Days*		
C 20	6–8[1]	2–5	1			115	404	1– 5–25	Totally
C 21	6–8[2]	5	1			127	351	11–30–24	Partially
C 112[3]	8–10[3]		Viosterol 20[4] drops			94	532	5– 1–32	Partially
C 32	Corn dis. grain	Corn gluten	Wheat straw	12–16	3–5	109	413	9– 8–25	Partially
C 44	0.5	2–3	ad lib.	12[5]	2–2.5	89	347	3– 9–26	Totally
C 45	0.5	2–3	ad lib.	12[6]	2–2.5	120	293	1–16–26	Totally
C 43	Cottonseed meal 2–6		ad lib.		2–4	94	292	10–31–25	Totally
C 49	3			6[7]	2–3	91	189	11–16–25	Partially
C 80	2–5		1–3	12[8]	1–2	94	311	7– 1–29	Partially
C 91				12	4–5	98	354	1– 1–31	Totally

[1] 150 pounds hominy
100 pounds corn distillers grains
50 pounds wheat straw
25 pounds cottonseed meal
17 pounds yeast
2 pounds sodium chloride
1 pound sodium phosphate
1 pound di sodium phosphate
0.5 pound magnesium phosphate
0.5 pound magnesium carbonate
0.25 pound potassium chloride
0.25 pound potassium carbonate
20 grams iron lactate

[2] 100 pounds grain mixture [1] plus 3 pounds calcium carbonate

[3] 50 pounds yellow corn
20 pounds oats
20 pounds yellow corn gluten meal
8.5 pounds linseed oil meal
1 pound calcium carbonate
0.5 pound sodium chloride

[4] 250 D.

[5] To 189 days of age.

[6] To 187 days of age.

[7] To 133 days of age.

[8] To 206 days of age.

[9] No sunlight.

Various types of rations were used which produced blindness in young growing dairy animals, as shown in table 5. The optic nerves of C 43, C 44, C 80, C 91 and C 112 were dissected out and showed the characteristic atrophy where they passed through the bony canal. Figure 6 shows the typical condition of the nerves of C 91.

Weekly or bimonthly blood calcium and inorganic phosphorus values were obtained on the dams of all the blind calves, except animals 200, 201 and 205. Some blood determinations were also made on a few of the blind calves at the time of birth. In many cases plasma chloride, carbon dioxide and hemoglobin determinations were made. These data, however, do not reveal any outstanding abnormalities. Similar data were obtained on the young growing dairy animals shown in table 5, but the data did not reveal anything which could be correlated with the development of blindness in every case.

DISCUSSION

The evidence presented indicates that corn silage, timothy hay and cod liver oil contain some factor or factors which prevents blindness in calves due to constriction of the optic nerves. From the cases thus far studied, it is evident that when the dams receive a ration with a roughage of poor quality, the blindness in their calves is due to atrophy of the nerve at the place where it passes through the bony canal. It also seems plausible from the evidence obtained in sectioning the nerves in the bony canal in three cases, and observing the small opening through which the nerve must pass in other cases, that the atrophy could be due to the pressure of the bone upon the nerve (fig. 4). It might be argued that the nerve first degenerated and that the canal through the bone did not enlarge as growth proceeded. It is difficult to conceive, however, that the degeneration of the optic nerve, if it were due to causes other than pressure, would always occur at the same place.

Examination of the bones from several cases did not reveal any exostosis. The bony canal gave more the appearance

of having had pressure applied from above which caused it to become smaller as growth proceeded. Due to the position of this particular structure, this would be the case if sufficient intracranial pressure were developed. The only evidence of intracranial pressure was that the animals were often subject to spasms on the slightest provocation. Furthermore, this type of blindness has never been noted to develop after the animal had reached a mature age, suggesting that sufficient calcification of the structures had probably taken place to resist any pressure.

Attention is also called to the possibility of a disturbed mineral metabolism related to vitamin D and rickets or to a low calcium ration. Examination of the blood calcium and inorganic phosphorus values did not reveal any correlation between blindness, low blood calcium, and inorganic phosphorus which would indicate that the animals were not suffering from rickets. All the animals were turned out to sunshine, except where indicated, yet blindness seemed to occur in almost any season of the year. When the animals were kept inside, they received ultraviolet light, viosterol or cod liver oil as a source of vitamin D, except in the case of C 75. X-rays of the ribs of many of the animals did not show a rachitic condition. The ash and mineral analyses of some of the bones were also made, but they did not reveal a rachitic condition. Therefore, it would seem doubtful that vitamin D was directly concerned in the cause of this type of blindness. The rations fed to the animals listed in tables 1 and 2 are low in calcium, but the rations fed to those listed in table 4 contained ample calcium.

Although no direct evidence has been produced, Hart and co-workers ('24), Halverson and Sherwood ('30) and Converse and Meigs ('32) believe that vitamin A is concerned in this problem where they report poor reproduction and blindness on rations with a poor quality of roughage. The type of blindness herein reported is not characterized by the external eye lesions associated with vitamin A deficiency, as reported by Mead and Regan ('31), Jones, Eckles and Palmer

('26) and Hart and Guilbert ('33). It seems doubtful that vitamin A could be concerned with such bone malformations as in the cases reported in this paper, but such an explanation might be plausible if the absence of vitamin A, in some indirect way, raised the intracranial pressure. Most of the rations used contained variable amounts of vitamin A from yellow corn. The rations shown in table 4 contained 50 per cent yellow corn and 20 per cent gluten meal. Corn gluten meal contains considerably more vitamin A than yellow corn. Even with the addition of 20 cc. of cod liver oil to the rations of C 103 and C 108 blind calves were produced. In every case where normal calves were produced on these rations, however, vitamin A had been added, so that it cannot be definitely ruled out. It is doubtful whether any of the rations contained as much vitamin A as is contained in 6 pounds of good quality alfalfa hay. Work is now in progress in which vitamin A in the form of carotene is being added to the ration in amounts equivalent to the vitamin A content of 10 pounds of alfalfa hay.

The question of storage of the dietary factor or factors concerned in this type of blindness must also be considered. Two mature cows from the regular dairy herd, not previously mentioned in this paper, and pregnant for 6 weeks, were placed on a ration of skim milk, corn and oats with shavings for roughage. These cows had normal calves at the end of a proper gestation period. C 83 (table 3) apparently received a sufficient amount of the factor or factors from 6 weeks on pasture at the beginning of the second gestation period to produce a normal calf.

This type of blindness is usually associated with poor reproduction, such as weak premature calves and frequently retained placentas. The calves were usually paralyzed in the front quarters, were too weak to suckle and sometimes exhibited spasms. The blindness may not always be associated with poor reproduction. However, O 1's, C 108's and C 35's first calves were fairly strong and active, although they were carried only 270, 267 and 258 days, respectively.

The blindness, whether it is present in the calf following birth or in the young growing dairy animal, appears to be due to identical factors, since in both cases there is a constriction of the optic nerve at the place where it passes through the bony canal. This type of blindness is more easily produced in calves during the intra-uterine period than in young growing dairy animals. It can be produced regularly in calves in utero provided the storage of the factor or factors is exhausted before gestation sets in, but it cannot be produced regularly in young growing dairy animals. Probably with more information concerning the factors involved, it can be produced with more regularity in young growing animals.

De Schweinitz ('31) and De Schweinitz and De Long ('34) report some excellent work on blindness in Guernsey calves apparently due to papilledema. Evidently they have not observed cases of constriction of the optic nerves as reported in this paper. They do discuss blindness, reported by others, due to atrophy of the optic nerve, which is apparently identical with the type of blindness reported in this paper, and show a photograph (fig. 16 in their last paper). The type of blindness which they have noted may be a different stage of the type herein reported. De Schweinitz indicated the possibility that the blindness observed in his cases may be inherited. In the work reported in this paper all of the cases have been produced in Holstein calves and we have never noticed blindness in the herd unless roughage was a limiting factor. Furthermore, this type of blindness is associated with paralysis and weakness at birth and poor reproduction, which De Schweinitz has apparently not noted in his cases.

Crocker ('19) described what is apparently the same type of blindness reported in this paper. He reported that the bone grew in such a manner as to stenose the canal and pressed the optic nerve within it.

The symptoms of cottonseed meal injury in cattle appear to be identical with the symptoms associated with this type of blindness. There is also the possibility that the curative factor or factors are identical. The evidence presented in

this paper indicates that a deficiency of vitamin A in the ration may not be the causative factor involved in cottonseed meal injury.

The authors have not observed blindness among calves under farm conditions. There is a possibility, however, during years of hay shortage, that cattle may suffer due to a lack of the factor or factors carried by hay which are necessary for good reproduction and health.

SUMMARY AND CONCLUSIONS

1. Twenty-four cases of a nutritional type of blindness have been described which are apparently different from the true vitamin A type of blindness.
2. The blindness is observed in calves following birth and in young growing dairy animals when a ration containing poor quality roughage has been fed.
3. It is characterized by a dilated pupil and by the absence of inflammation of the external structures of the eye.
4. It is frequently associated with paralysis, weakness, spasms and poor reproduction denoted by premature births and retained placentas.
5. The blindness is due to atrophy of the optic nerve where it passes through the optic foramen, apparently because of bone pressure.
6. A deficiency of vitamin A may or may not be the cause of this type of blindness.
7. The evidence indicates that vitamin D is not the major factor in this type of blindness as the blood picture is not significantly different from normal.
8. The evidence further indicates that a deficiency of calcium is not directly concerned in this type of blindness.
9. Corn silage, timothy hay and cod liver oil contain the factor or factors necessary to prevent this type of blindness.

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INTESTINAL STASIS IN LOW MINERAL DIETS

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FOUR FIGURES

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In the course of experiments on the resistance of rats fed a mineral poor diet to oral infection with a paratyphoid bacillus, some of the rats were given carmine in order to learn how soon the organisms were excreted. It was noticed that the rats passed carmine-colored feces for many days and it was thought advisable to investigate this intestinal stasis further.

ANIMALS AND BASAL DIETS

Litters of albino rats were divided a day or two after weaning into two or more groups. One group was then fed an adequate diet, and the other group was given the same diet minus the salt mixture and NaCl. The two diets were constituted as follows:

	Adequate diet Per cent	*Mineral deficient diet* Per cent
Cornstarch	59	64
Purified casein (treated with dilute acetic acid and also hot alcohol)	18	18
Crisco	10	10
Cod liver oil	2	2
Dried brewer's yeast	6	6
NaCl	1	..
Osborne and Mendel's salt mixture	4	..

The mineral deficient diet was not mineral free, as the casein contained some P and Ca, and the yeast contained a

total ash of about 5.6 per cent. The Ca, P, Fe and Cu contents of the diets were determined and were found to be as follows:

Elements	*Mineral deficient diet*	*Adequate diet*
Ca	0.0794 gm. per cent	0.4294 gm. per cent
P	0.1176 gm. per cent	0.3276 gm. per cent
K	Not determined (call x)	x + 0.52 per cent
Fe	0.0062 gm. per cent	0.0252 gm. per cent
Cu	A trace	A trace

As is shown in figure 1, the rats fed the mineral deficient diet grew very slowly, whereas those fed the adequate diet

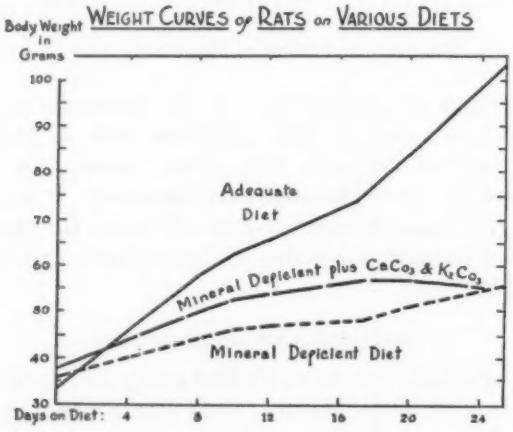

Figure 1

increased rapidly in weight. The animals that had been kept on the deficient diet for 4 weeks often squeaked a great deal when touched, frequently bit anyone who handled them, which is very unusual with our normal rats, and sometimes dragged their hind legs, although they were still able to move them.

A few bone ash determinations and x-ray photographs were made which showed that the bones of the rats fed the mineral poor ration were osteoporotic and low in ash, whereas those of the controls were normal. The polycythemia which Swanson and Smith ('32) observed in rats fed a diet very deficient in minerals was confirmed.

Method of administering carmine. After a certain period on the diet, usually 4 weeks, the rats were starved for 16 to 17 hours and then fed by pipette about 6 mg. of carmine in 0.2 cc. of simple syrup. The same batch of carmine was used throughout. The rats were kept in individual wire mesh bottomed cages, usually for some time before feeding the carmine, and the feces were collected on paper which was placed beneath the mesh out of reach of the rats.

The first collection of feces was made 24 hours after the carmine feed and from then on daily. The pellets were examined in a little water and crushed with forceps, and if the distinctive red color was not evident they were shaken up with 3 to 4 cc. of water. This suspension sometimes appeared pink when no carmine had been seen in the individual pellets. If the carmine could not be positively detected by these means, the suspensions were examined under the microscope, using the high dry lens. Under this magnification the carmine showed up very brilliantly as cerise particles with sharp corners. When no carmine was found, at least three big drops were examined, and the carmine was not considered entirely excreted unless two such examinations at 24-hour intervals were negative. In a few animals the feces were further examined for several more days and were found to be free from carmine, thus establishing the accuracy of the end point.

We have found no evidence as yet, either by examining the mesentery under the microscope or by cutting open the kidneys, that carmine is absorbed from the gut, and the urine apparently did not contain any of it.

Experiment 1. Eight rats were placed on the mineral deficient diet and eight on the adequate diet and after 4 weeks, when the former weighed on the average 75 gm. and the latter 112 gm., the rate of excretion of the carmine was determined. The results are shown in figure 2. The carmine appeared 24 hours after it was administered, and the rats fed the adequate diet excreted carmine for from 3 to 7 days, whereas those fed the mineral deficient ration showed carmine in their feces for from 12 to 21 days. The fecal pellets of those fed the

deficient diet at all times contained a lower concentration of carmine than those of the animals fed the adequate diet. The marked intestinal stasis shown by the rats fed the mineral deficient diet has been confirmed in over seventy-five other rats. A corresponding number of adequately fed controls failed to show the stasis.

Experiment 2. Three rats were kept on each of the two diets for 4 weeks and at the end of this time when those on the

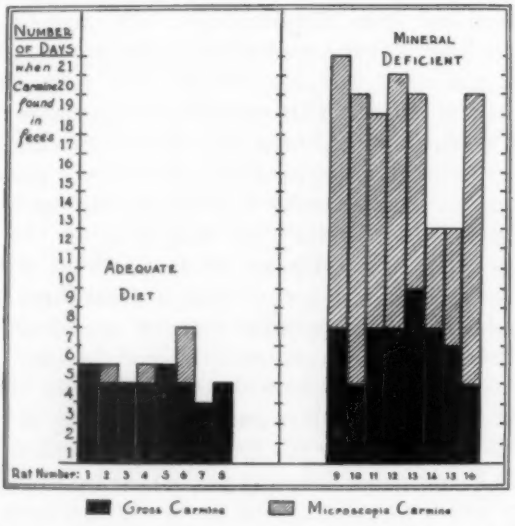

Fig. 2 The much slower excretion of carmine by rats fed the mineral deficient diet as compared with those fed the adequate diet.

adequate diet weighed 135, 117, and 112 gm., respectively, and those on the mineral deficient diet 58, 52 and 47 gm., they were fed the carmine. The rats on the adequate diet excreted carmine for 3 to 5 days, and those on the mineral deficient diet for 10 to 17 days (fig. 3, columns 1, 2, 3 and 7, 8, 9).

In this experiment the weight of food eaten and of the feces excreted daily by each rat during the period in which the carmine was being excreted was also determined. The feces

were dried for 48 hours on a hot radiator before they were weighed. The results are shown in table 1.

The food intake and the weight of the feces of rat 141 were low, because its water bottle did not run properly from the third to the fifth day of the test. Despite this fact it eliminated the carmine as rapidly as rat 140 which was fed the same diet.

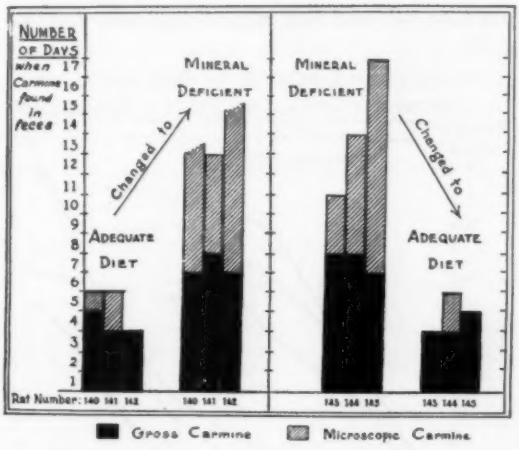

Fig. 3 The effect of alternating the deficient and the adequate diets on the rate of excretion of carmine.

TABLE 1

RAT NO.	DIET	NUMBER OF DAYS CARMINE EXCRETED	TOTAL FOOD EATEN WHILE EXCRETING CARMINE	TOTAL DRIED FECES CONTAINING CARMINE
			gm.	*gm.*
140	Adequate	5	61.5	2.177
141	Adequate	5	36.9	1.051
142	Adequate	3	42.1	1.205
143	Mineral deficient	10	68.6	3.446
144	Mineral deficient	13	87.8	1.905
145	Mineral deficient	17	84.7	1.807

The daily food intake of the rats fed the mineral poor ration was, as one would expect from their much smaller body weights, somewhat less than half of that eaten by the rats on the adequate diet. One might guess that it would take

the same amount of food to carry away all of the carmine no matter what the diet was. Such, however, does not follow from this experiment as the rats fed the mineral deficient diet had to eat a great deal more food and excrete more feces before they got rid of all the carmine than did those fed the adequate ration.

Experiment 3. Effect of alternate deficient and adequate diets. Rats 140, 141 and 142, which had been fed the adequate

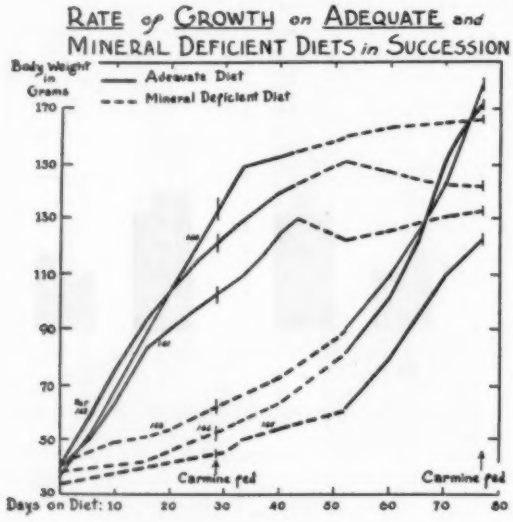

Figure 4

diet for 43 days, were placed on the mineral deficient diet, and after 34 days on it, when they weighed, after 18 hours' starvation, 159, 136 and 137 gm., they were fed carmine again. Similarly, the rats which had been fed the mineral deficient diet for 52 days were changed to the adequate diet, and after 25 days on it, when they weighed 171, 163 and 113 gm. (starved), they were again fed carmine. All of these rats were consequently of approximately the same weight. The weight curves of these rats are shown in figure 4. Even after

52 days on the deficient diet, during which time very little growth occurred, the subsequent feeding of the adequate diet led to rapid gains (rats 143, 144, 145). The rates of excretion of the carmine after the diets had been changed are shown in columns 4, 5, 6 and 10, 11 and 12 of figure 3. Marked stasis was shown by the rats, formerly on the adequate diet, which had been fed the deficient diet for 5 weeks, and no stasis in the rats which had been on the adequate diet for 4 weeks, although they had previously shown stasis on the deficient diet. Unfortunately, the exact day on which rats 140 and 142 stopped excreting carmine was not determined. It was some time after 12 and 14 days. The results of this experiment rule out the factor of body weight, as the stasis shown

TABLE 2

Carmine excretion

RAT NO.	ON MINERAL DEFICIENT DIET		AFTER 7 DAYS ON ADEQUATE DIET	
	Gross	Microscopic	Gross	Microscopic
	Days	*Days*	*Days*	*Days*
17	8	10	3	7
18	7	11	3	6

by rats 140, 141 and 142 (on mineral deficient diet) was as great as usual, although they weighed as much as the adequately fed animals. This experiment also shows that the deleterious effects of the mineral poor diet did not permanently injure the rats.

Experiment 4. The above experiment was repeated in a somewhat modified form. The rate of excretion of carmine was determined in two rats which had been fed the mineral deficient diet for 5 weeks. After 3 weeks more on the deficient diets the rats were given the adequate diet for 1 week and carmine was again fed.

The results of this test in table 2 show that the stasis is much improved, although not entirely relieved by 1 week's feeding of the adequate diet.

Experiment 5. To further test out the effect of body weight on the rate of excretion of the pigment, we fed carmine to fifteen young rats which had been on the adequate diet for a week and which weighed about as much as rats that had been fed the mineral deficient diet for 4 weeks. No macroscopic carmine was seen in this lot after 3 days, and in four rats it was not evident to the naked eye after only 2 days. When the feces were examined through the microscope, carmine was usually not found for more than 4 days after the carmine feeding. Occasional rats were free after 2 or 3 days, and three showed carmine on the fifth or sixth day. In other words, small normal rats excrete the carmine as rapidly or possibly more rapidly than the large normal animals and the slower elimination in the mineral deficient rats of experiment 1 was not due to their small size. These results do not correspond with those of Gross ('24), who suggested that small rats excreted charcoal more slowly than large rats.

Experiment 6. As a lack of the vitamin B complex causes intestinal stasis, it was thought that possibly the rats fed the mineral deficient diet were not able to utilize this vitamin, which was given as 6 per cent dried yeast, as well as the adequately fed controls. Therefore, 66 mg. of a vitamin B concentrate made from yeast and wheat germ was fed to the rats in addition to the usual mineral deficient and adequate diets. This amount of concentrate should contain, according to the manufacturers,[1] twice as much vitamin B_1 and as much vitamin B_2 as the rats should require daily. After 4 weeks on the diets, when the adequate rats weighed 100, 146, 80, 110 and 119 gm., and the mineral deficient 112, 55, 75 and 85 gm., the carmine was fed.

From table 3 it is seen that the rats fed the deficient diet with the additional vitamin B concentrate excreted the carmine just as slowly as the deficient rats of the preceding experiments.

The concentrate contained no Ca, Fe or Cu, but there was about 0.4 mg. P in the amount given daily. The amount of phosphorus in the diet was, therefore, almost doubled (raised

[1] Mead Johnson & Co.

to 0.218 per cent) without improving the stasis. It is doubtful, therefore, if still further additions of phosphorus would improve the elimination.

Experiment 7. Although it was not suspected that Osborne and Mendel's salt mixture would have any laxative effect, it was thought wise to substitute another standard salt mixture. Therefore, in this experiment the rats fed the adequate diet

TABLE 3

The effect of the addition of vitamin B concentrate. Carmine excretion

ON MINERAL DEFICIENT DIET PLUS VITAMIN B COMPLEX			ON ADEQUATE DIET PLUS VITAMIN B COMPLEX		
Rat no.	Gross carmine	Microscopic carmine	Rat no.	Gross carmine	Microscopic carmine
	Days	*Days*		*Days*	*Days*
33	7	10	28	3	5
34	11	20	29	4	4
35	8	11	30	4	6
36	11	17	31	4	6
			32	4	4

received 4 per cent of McCollum's salt mixture no. 51, which contains the following ingredients:

	Grams
$CaCO_3$	15
K Cl	10
NaCl	5
$NaH\ CO_3$	7
Mg O	2
Ferric citrate	5
KH_2PO_4	17

It contains no S, I, Mn, F and Al which are all present in the Osborne and Mendel salt mixture. The mineral deficient diet remained as before. The evacuation of the carmine was just as rapid in the rats fed the adequate diet containing the no. 51 salt mixture as in the rats fed the original adequate diet. One would therefore infer that a deficiency of S, I, Mn, F or Al was not responsible for the stasis. It was also found that Osborne and Mendel's salt mixture in 3 iii to 3 iv doses was not laxative for an adult woman.

Experiment 8. The 1 per cent of NaCl was left out of the adequate diet in one experiment, and after 4 weeks on this diet the carmine excretion was tested as before. The rats fed this diet excreted the carmine as rapidly as the controls which were fed the usual adequate diet. Consequently it would appear that the presence of NaCl does not affect the intestinal motility.

TABLE 4

Adequate diet for 8 weeks

FED CARMINE AND KILLED AFTER	RAT 1	RAT 2	RAT 3	RAT 4	RAT 5
	24 hours	48 hours	72 hours	96 hours	168 hours
Stomach	—	⊕	—	—	—
Duodenum	—	—	—	—	—
Jejunum	—	—	—	—	—
Ileum	—	—	—	—	—
Cecum	+++	+	—	—	—
Colon	++++	+	⊕	—	—

Mineral deficient diet for 8 weeks

FED CARMINE AND KILLED AFTER	RAT 6	RAT 7	RAT 8	RAT 9	RAT 10
	24 hours	48 hours	72 hours	96 hours	168 hours
Stomach	+++	++	+	++	—
Duodenum	—	+	—	—	—
Jejunum	++	+	+	++	—
Ileum	+++	++	+	++	—
Cecum	++++	+++	++	+++	⊕
Colon	++++	+++	++	+++	⊕

+ = Gross carmine. ⊕ = Microscopic carmine only.

Experiment 9. In this experiment rats which had been fed the regular adequate diet, and others which had been given the mineral deficient diet for 8 weeks were fed carmine and then one of each group was killed daily and the intestines were examined for carmine. The results are shown in table 4. The carmine was rapidly moved through the intestinal tract by the rats subsisting on the adequate diet. On the other hand, 4 days after the administration of the carmine the rats fed the deficient diet showed macroscopic carmine in con-

siderable quantities all through the intestine. After 7 days there was more carmine in the rat fed the mineral poor ration than in the rat on the adequate diet that was killed 4 days previously. This experiment again shows the marked stasis present in the rats fed the mineral deficient diet. The stasis seems to involve the whole intestinal tract, although it is especially evident in the cecum and colon.

Weight of the intestines and contents. Rats which had been fed the diets for varying periods of time were killed by ether, and after a ligature of thin thread had been put around the pylorus and another around the ileocecal valve, the whole intestine with as little mesentery as possible was removed

TABLE 5

Average weights of intestines and contents

BODY WEIGHTS	ON MINERAL DEFICIENT DIET			ON ADEQUATE DIET		
	Cecum and colon	Total intestines	Number of guts weighed	Cecum and colon	Total intestines	Number of guts weighed
gm.	*gm.*	*gm.*		*gm.*	*gm.*	
51– 60	1.704	4.378	1	1.583	4.692	1
61– 70	2.544	5.672	4	1.642	5.609	7
71– 80	2.694	5.990	4	1.694	5.196	2
81– 90	2.412	6.204	4	1.953	5.979	3
91–100	2.690	6.659	3	....	....	..
101–110	2.782	6.680	1	1.939	5.216	1

and weighed at once. The small intestine was then weighed separately.

When the average total intestine weights of rats of closely similar body weights (table 5) were compared, it was found that those of the rats on the mineral deficient diets were heavier than those of the animals on the adequate diets, with the exception of the two smallest rats. The cecums and the colons weighed considerably more in the rats on the deficient diet in every case. In other words, definite stasis was evident in the cecum and colon of the rats fed the mineral poor diet. The difference is probably greater than is evident from these figures, as the weight of the intestinal wall is probably about the same in both groups of rats and is responsible for a large

part of the total weight. As the food normally passes very rapidly through the small intestine, one would expect a priori that the stasis would occur in the large bowel.

Gross appearance of the intestinal tract. The guts of the rats which were killed in experiment 9 were briefly examined with the naked eye for gross abnormalities. No definite dilation was seen in the upper intestines or the cecums of the rats fed the mineral deficient diet. The colons of the deficiently fed rats were, however, more dilated than those of the controls, the walls of the colons were also thinner and more friable, the haustra were more prominent especially near the cecum, and the fecal matter was more plentiful and softer. This increased size of the colons was all the more remarkable when one noted that all the rats fed the mineral poor diet, except the first one killed, weighed about half as much as the rats on the adequate diet.

Attempts to alter the rate of excretion by adding various elements

In these experiments, which are not yet completed, rats were fed the mineral deficient diet with the addition of various salts for 4 weeks and after that interval the rate of excretion of the carmine was determined. Control rats fed the mineral deficient and the adequate diet were included in each experiment.

The addition of 1.1 per cent NaCl to the mineral deficient diet, which provides about as much NaCl as is present in the adequate diet, caused very little change in the rate of excretion; in a few rats it was a little faster, in others it was somewhat slower.

When 1 per cent of $CaCO_3$ was added to the mineral deficient diet, very little if any improvement in the intestinal stasis occurred. The diet after this addition contains 0.479 per cent of calcium, which is slightly higher than that of the adequate ration.

At present the effect produced when 1 per cent K_2CO_3 is added to the mineral deficient diet is being investigated. The

results to date have been contradictory. This amount of K_2CO_3 compensates for the K present in the adequate diet.

When 1 per cent $CaCO_3$ and 1 per cent K_2CO_3 were added to the deficient ration, the rats excreted the carmine as rapidly as the controls fed the adequate diet. This experiment has been repeated four times and all of the twenty-seven rats which were fed this diet showed no stasis. When 0.17 per cent $MgCO_3$ was added as well, to compensate for the Mg present in the adequate diet, the results were the same as when both $CaCO_3$ and K_2CO_3 were added, i.e., there was no stasis.

The total weights of the intestines and contents of the rats fed the mineral deficient diet + $CaCO_3$ + K_2CO_3 were decidedly less than those of rats fed the deficient diet alone, and also less than those fed the adequate ration. This adds confirmation to our previous finding that the simultaneous addition of $CaCO_3$ and K_2CO_3 to the mineral deficient diet relieves the stasis.

In order to determine whether K_2CO_3 had a laxative effect per se, 1 per cent of this salt was added to the adequate diet. This approximately doubled its K content. The rats fed this ration excreted the carmine at the same rate as rats fed the regular adequate diet. In other words, the K_2CO_3 did not act as a laxative. These findings were confirmed in a second test. When the amount of the salt mixture was raised to the high amount of 8 per cent the carmine was excreted a little more rapidly than it was by the controls fed the usual adequate diet.

In the course of these experiments there was some evidence that hot weather leads to a decrease in the speed of excretion, both in rats fed the adequate diet and in those fed the mineral poor ration. This is being checked further.

DISCUSSION

The mineral poor diet used in these experiments had in all probability a much lower mineral content than even the poorest human dietaries, but it is possible that the long-continued

consumption of a diet partially deficient in these factors may also lead to intestinal stasis. Constipation is a very prevalent malady among civilized peoples, and it is also a fact that many of the highly processed food-stuffs which are much favored, such as white flour and sugar, are very low in minerals. Approximately 50 per cent of the total calories in an average diet are derived from these two sources, and in the diet of the poor the percentage is often higher. In addition, the common method of boiling vegetables in which the cooking water is discarded no doubt leads to great losses of the mineral salts, especially of those that are highly soluble, such as potassium. Keller ('34) states that from three-quarters to five-sixths of the salt content of thoroughly boiled potatoes is lost when the water in which they are cooked is thrown away. Over indulgence in table salt, which is a fairly common habit, also increases the potassium loss. The combination of all these factors may perhaps play a part in the production of constipation.

In these experiments we have not yet established conclusively that both potassium and calcium salts must be added simultaneously to the deficient diet to prevent the stasis that would otherwise occur, but this would appear to be the case. It is interesting to note that numerous investigators working on other physiological problems have emphasized the importance of the K/Ca balance in maintaining the well-being of animals. On looking through the analyses of foods, one is struck by the fact that practically all of the laxative foods fed to young children are very high in potassium. Many of them contain much roughage, but this is not always the case, as, for example, with prune juice. It is not impossible that this high potassium content is a factor in their laxative effect, especially as the calcium intake is high. Again, it is well known that human milk is more laxative than cow's milk. This may possibly be due to its higher K/Ca ratio (v. Wendt, '25). Further work is being done on these very practical problems.

SUMMARY

1. Young rats fed a diet low in minerals, but adequate otherwise, show a great delay in the excretion of 6 mg. of carmine. In other words, they have a marked intestinal stasis. Control animals fed the same diet plus the minerals do not show this stasis.

2. Additional vitamin B complex does not relieve the stasis of the mineral deficient animals.

3. The simultaneous addition of $CaCO_3$ and K_2CO_3 to the mineral poor ration seems to relieve the stasis.

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A COMPARISON OF THE ANTIRACHITIC POTENCY OF COD LIVER OIL AND IRRADIATED ERGOSTEROL ON A CURATIVE AND PREVENTIVE BASIS [1]

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It is well known that antirachitically equivalent amounts of the antirachitic factor in the form of cod liver oil and irradiated ergosterol, as measured by a curative assay with the white rat, are not equally effective when used in a preventive manner with the chicken (Massengale and Nussmeier, '30; Mussehl and Ackerson, '30; Steenbock et al., '32; Bethke et al., '33, and unpublished data from this laboratory). The purpose of this investigation was to compare the antirachitic potency of cod liver oil and of irradiated ergosterol upon a curative and preventive basis, using the white rat as the test animal. Such a comparison was of interest in connection with investigations of these two sources of the factor when the chicken was used as the experimental subject. Studies with the chicken have been made, in practically every instance, upon a preventive basis and the quantities of cod liver oil and irradiated ergosterol used have been determined by curative assays made with the white rat. Hence the question was raised as to whether a preventive assay of both sources of the factor, made with the rat, would show results in agreement

[1] Journal series paper of the New Jersey Agricultural Experiment Station, Department of Agricultural Biochemistry.

[2] Acetol Products Fellow. This investigation was conducted under a fellowship grant from Acetol Products, Inc., New York.

with a curative assay with this species, or whether they would be in agreement with the results of preventive studies with the chicken.

EXPERIMENTAL

For the preventive studies young rats, 55 to 60 gm. in weight, were placed upon the experimental regime at 23 to 28 days of age. Litter representatives were assigned to each of the two groups receiving the two sources of the factor. The supplements listed in table 2 were incorporated in 50 gm. of the Steenbock 2965 ration as in a curative assay. Successive 50-gm. portions were supplied throughout the experiment, a portion being consumed in about 7 days. After 40 days of feeding, the blood was drawn from the heart, under anesthesia, for the determination of inorganic P (Briggs, '24) and the femurs removed for ash determinations. Individual determinations were made of P in whole blood and of bone ash of the right femur (Russell and Massengale, '28).

The curative assay, using the line-test method, was conducted essentially as outlined by the Wisconsin Alumni Research Foundation. Young rats of the same age and weight range as those employed for the preventive study were depleted in 23 or 24 days and then carried through the 10-day test period. The supplements were mixed with 50-gm. portions of the Steenbock 2965 ration, a portion being consumed during the first 6 or 7 days of the test period.

DISCUSSION

In table 1 the line test responses at the unit levels are shown for the two sources of the antirachitic factor, at the beginning and at the end of the preventive experiment. The unit amounts or multiples thereof were used in the preparation of the 50-gm. portions of supplemented ration in the preventive study.

A comparison of the bone ash percentages, table 2, reveals no significant difference between the responses to the two sources of the antirachitic factor. The value, 38.43 per cent, for 0.5 unit of irradiated ergosterol is lower than that for

TABLE 1

Curative assay of irradiated ergosterol and cod liver oil. Responses at unit levels

DATE OF ASSAY	IRRADIATED ERGOSTEROL		COD LIVER OIL	
	Unit level	Response	Unit level	Response
	mg.		*mg.*	
October, 1932	0.0008	+(++) +, +, +, + ± −, −	45	+(++), +(++) +, +, + ±, ±
August–September, 1933	0.0008	+++ +(++), +(++) +, +, +, + ±	45	++ +(++), +(++) +, +, + ±(+) ±

NOTE: + equals a narrow continuous line. The unit level is the smallest quantity of material which causes 60 per cent of the responses to be + grade or slightly better, including one-half of those of ±(+) grade.

TABLE 2

Comparison of irradiated ergosterol and cod liver oil on a preventive basis. Summary of food consumption, gains in weight, blood inorganic phosphorus values and bone ash percentages. Duration of test period—40 days

SUPPLEMENT IN 50 GM. OF FOOD	RAT UNITS. CURATIVE BASIS	NUMBER OF ANIMALS	AVERAGE DAILY FOOD CONSUMPTION	AVERAGE DAILY GAIN IN WEIGHT	INORGANIC P OF WHOLE BLOOD			BONE ASH OF RIGHT FEMUR		
					Per 100 cc.	Probable error	Coefficient of variation	Value	Probable error	Coefficient of variation
mg.			*gm.*	*gm.*	*mg.*		*per cent*	*per cent*		*per cent*
Irradiated ergosterol										
0.0004	0.5	10	6.5	1.2	2.2[1]	± 0.036	7.29	38.43	± 0.79	9.76
0.0008	1.0	10	7.5	1.3	2.9[1]	± 0.036	5.45	43.80	± 0.74	7.98
0.0016	2.0	10	7.9	1.4	3.0	± 0.041	6.33	46.76	± 0.39	3.86
0.0080	10.0	8	7.6	1.3	3.5	± 0.035	3.90	51.18	± 0.43	3.60
Cod liver oil										
22.5	0.5	10	7.8	1.5	2.6[1]	± 0.044	5.09	41.38	± 1.00	11.40
45.0	1.0	10	7.5	1.4	3.2	± 0.053	7.78	44.26	± 0.79	9.76
90.0	2.0	10	7.5	1.3	3.5	± 0.050	6.71	46.15	± 0.74	7.98
450.0	10.0	8	7.9	1.4	3.7	± 0.029	3.26	51.80	± 0.33	2.66
Controls										
No supplement	....	9	6.2	0.9	1.4[2]	±	....	24.48	± 0.56	10.20

[1] Nine determinations.

[2] Six determinations.

the equivalent amount of cod liver oil, but it should be noted that the average daily intake of the irradiated ergosterol lot was 13 per cent less than the average daily intake of the other lots which received this form of the factor. Hence the actual intake was 0.43 unit rather than 0.5. This difference could account for the lower value in the case of this level.

If there were a difference in the response to the two sources of the antirachitic factor, it should have been evident at the 0.5-, 1.0- or 2.0-unit levels, because none of these showed the maximum bone ash percentage which was obtained when 10 units were fed. According to Hume and her associates ('32), 47 to 48 per cent bone ash may be the maximum for a diet low in phosphorus, and Dutcher, Creighton and Rothrock ('25) obtained 52.0 per cent bone ash with the Steenbock 2965 ration plus irradiation, at approximately the same age at which the animals were killed in this experiment. Although higher levels were not fed, in the light of the results of the above investigators, it would seem that 51.18 per cent and 51.80 per cent for the irradiated ergosterol and cod liver oil groups, respectively, at the 10 unit level are maximum values for this ration and age of animal.

Four hundred and fifty milligrams of cod liver oil, 10 rat units, per 50 gm. of ration is equal to 0.9 per cent of the ration. Therefore, the 10-unit level of irradiated ergosterol, which produced the same maximum bone ash response, is equal in its effect on the rat to 0.9 per cent cod liver oil. In the case of the chicken, practically the same percentage of the same sample of cod liver oil, 1 per cent, or 22 rat units per 100 gm. of ration, was four times the amount necessary to produce a maximum bone ash value, but the quantity of the same sample of irradiated ergosterol required to produce a maximum response was 192, or possibly 144 times, the antirachitic value of the cod liver oil at the minimum protective (0.25 per cent) level (unpublished data).

The inorganic P of the whole blood is highest for the groups which received 10 units per 50 gm. of diet and the values for the others became less as the allowance of both forms of the

factor decreased. In all instances the phosphorus values of the irradiated ergosterol groups are slightly lower than those of the corresponding cod liver oil groups. It is of interest that the coefficients of variation of the completely protected groups are low as compared with the values for the basal and partially protected groups.

The unit level determined by the usual 10-day curative assay with vitamin D-depleted animals does not afford complete protection when fed on a preventive basis. Schultzer ('31) has compared the preventive and curative methods, but the technics employed are so different from those used in this laboratory that a comparison of results is not possible. At a later date he reported ('33) the preventive dose to be equal to the curative. As indicated above, we have not found this to be the case, but again the methods employed by the two laboratories are so different that comparisons cannot be made.

SUMMARY

1. The response of the white rat to antirachitically equivalent amounts of cod liver oil and irradiated ergosterol, determined curatively, is the same when the two sources of the factor are fed on a preventive basis. This result is strikingly different from that obtained in the chicken, in which species the irradiated ergosterol form of the antirachitic factor is much less effective than the cod liver oil form when compared on a preventive basis.

2. The minimum protective dose on a preventive basis is between 2 and 10 curative units per 50 gm. of ration. The unit or 1+ level, determined curatively, affords only partial protection when fed on a preventive basis.

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STUDIES ON LACTATION

II. TECHNIC FOR STUDYING LACTATION IN RATS

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ONE FIGURE

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Most of the existing methods for studying lactation quantitatively are based on humans, cows, goats or dogs as experimental subjects. These methods are time consuming and costly when used in the study of the effects of isolated food substances upon lactation. However, they do afford the opportunity of making quantitative analyses of the milk. One must rely upon the growth rate of the suckling young or some other such factor when small animals like the rat are employed. Methods of this type have the advantages of being relatively rapid and less costly, even though the accuracy is not so great due to the use of indirect criteria of milk production. This inaccuracy can be lessened considerably by carefully controlled experimental conditions and the employment of good growth measurement methods. Methods heretofore used involved mortality and weaning statistics or percentage weight changes of the litter.

In the earlier work on lactation in small animals it was justifiable to use mortality statistics, percentage of young weaned, etc., but since it has become possible to rear rats on a practically synthetic diet, these criteria do not permit the finer discriminations between the effects of various food extracts and concentrates. If one young died in a litter of ten, the per cent weaned would be 90; but if one died in a litter

of two the per cent weaned would be 50. The average weaning weight and the per cent increase in weight over the suckling period are of little value, since they depend too largely upon the birth weight which in turn depends to some extent upon the number in the litter. The following table illustrates some of these points.

TABLE 1

NUMBER BORN	NUMBER WEANED	BIRTH WEIGHT OF SIX	WEANING WEIGHT OF SIX	PER CENT WEIGHT INCREASE
7	6	32	197	515
8	6	29	197	579

It is of primary importance to limit the litters to a definite number at birth. Some investigators limit the litters to four young, but most prefer six. Donaldson ('25) has pointed out that rats in relatively small litters grow much faster than rats in larger litters. Another means of lessening the inaccuracy of small animal methods is to disregard the growth data of the first 3 or 4 days of life. There is considerable variation of growth during this period, due to the dehydration of the young and the stabilization of the habits of the mother. It seems advisable, therefore, not only to limit the litters to six young, but to start plotting the growth data from the fourth instead of the first day of life.

Female rats vary somewhat in their inherent ability to mother a litter. This makes it necessary to exclude from the final data all but those experiments where the mother has successfully cared for and weaned the six young allotted to her at birth. It must be remembered that we are here dealing with a method to be used in the study of the augmentation of normal lactation with various food stuffs. We advise including only 'perfect' experiments in the final data, i.e., experiments where the mother has shown no signs of ill health and has successfully cared for all her young remaining after suitable reduction of the litter at birth.

Sugiura and Benedict ('18) have pointed out that a diet may be adequate for reproduction and growth of the young

after their eyes have opened, but not for the lactation period. This, then, limits the true lactation period of the rat to 16 or 17 days, since at this time the young open their eyes and begin nibbling at the mother's food. Many investigators have endeavored to prolong this true lactation period (the period over which the young are solely dependent upon the mother's milk for substance). They have devised ingenious food traps, etc. These have all proved unsuccessful, because of the manner in which a rat eats and because of the agility of the young at this age. Kozlowska, McCay and Maynard ('32) have even gone so far as to remove the mother from the cage at definite intervals for separate feedings. This, they admit, is rather impractical and too laborious. We feel that if the conditions are well controlled and only 'perfect' experiments are used in compiling the data, the growth periods from the fourth to the seventeenth day are of sufficient length to make comparisons.

Brody ('27) has analyzed Donaldson's growth data on white rats and finds that growth in the rat is definitely logarithmic and that the instantaneous percentage rate of growth remains constant for relatively long periods of growth. The rate is about 12 per cent for the first 10 days and about 4 per cent for the next 20 days. We have applied essentially his method to our data. Instead of using natural logarithms, we have used ordinary logarithms. The logs of the daily weights of the litters (six young are weighed together) from the fourth to the seventeenth day of life are plotted against time in days. Straight lines are then drawn through the greatest number of points. For this growth period (fourth to seventeenth day) there are two straight lines. The break occurs in the great majority at the tenth day. It may occur a day earlier or a day later. The majority of points fall on the two straight lines. Many other means of plotting and drawing lines were tried, but this method seemed to satisfy the conditions best. A numerical constant is obtained for the slope of each straight line by dividing the log difference between any two points on the line by the distance between

them in days. Both of these constants differ with a fair degree of significance when the diets of the lactating mothers differ. Figure 1 shows a compilation of the results obtained on a few different diets that are listed in table 2. The salt mixture used is shown in table 3. It is a slight modification of that used by Clayton ('30).

It is not within the scope of this paper to speculate upon the reason for the sudden change in the rate of growth of the white rat at approximately the tenth day of life. Suffice to

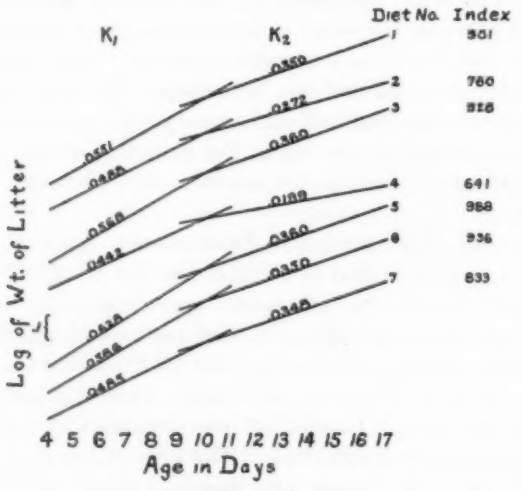

Figure 1

say that from a complete analysis of our data we find it occurring in about 90 per cent of the 300 litters studied. Brody gives no conclusive reason for it. It is our belief that it is characteristic of the species, although nutritional conditions may modify it.

In order to facilitate a quicker comparison of results, we have arbitrarily combined the two growth slope constants by simple addition and dropping the decimal point. The constants thus lose their actual mathematical significance and become merely round numbers to be used for comparison. We have chosen to call the resultant figure a lactation index.

We believe this method is a more accurate picture of the growth changes in the young due to lactation changes in the mother than is presented by any other existing means of judging lactation in rats. Its application and significance will be stressed in the following paper.

TABLE 2

Diets

	PARTS						
	1	2	3	4	5	6	7
Casein		15	25	15	12	15	Stock diet Purina dog chow
Cystine					1	1	
Glycine					1		
Glutamic acid					1		
Cod liver oil				14			
Liver (dried)	22						
Salt mixture	5	5	5	5	5	5	
Agar agar	2	2	2	2	2	2	
Starch	76	76	66	76	76	76	
Lard	14	14	14		14	14	

Wheat germ oil, 5 drops per rat per day on all diets.
Cod liver oil, 6 drops per rat per day on all diets.
Irradiated yeast, 3 gm. per rat per day on all diets.

TABLE 3

Osborne-Mendel salt mixture as modified

	gm.
$CaCO_3$	539.2
$MgCO_3$	96.8
Na_2CO_3	136.8
K_2CO_3 $1\frac{1}{2}$ H_2O	675.6
85% H_3PO_4	284.0
Conc. HCl	504.0
Conc. H_2SO_4	21.0
Citric acid (dry)	406.0
Iron citrate	25.36
KI	1.4
$MnSO_4$ H_2O	8.7
NaF	5.0
Alum	4.2
Na_2Si O_3 H_2O	25.0

SUMMARY

A method is described for the study of lactation in rats. It is dependent upon two growth constants derived from plotting the logs of the litter weights from the fourth to the seventeenth day of life against time in days. By combining the two constants a mathematical lactation index is obtained.

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STUDIES ON LACTATION

III. EFFECT OF VARIOUS DIETARY PRINCIPLES ON LACTATION IN RATS

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ONE FIGURE

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In these studies we are not primarily interested in mammary development, the initial stimulation of milk flow, or the transitory effects of drugs, vasodilators, etc., but rather in increasing the level of milk production already established and still more specifically in why adequate good quality protein maintains a high level of milk production.

Eckles ('16) stated that

> the error is often made of assuming that the richness of the milk varies with the feed. While it is possible under certain conditions to make a variation of possibly 0.2 to 0.4 per cent in the fat by giving certain foods, it is only under conditions so abnormal that it is of scientific interest only and has no practical bearing . . . . The richness of a cow's milk is fixed by heredity and cannot be permanently changed by any means.

It is quite true that the heredity factor, or the individual characteristic of the gland is the most important factor in milk production, but since Eckles made the above statement several workers have succeeded in materially altering lactation by dietary means, especially in rats. The most striking influence has been brought about by feeding a high protein diet. Class A animal proteins have been most successful in stimulating lactation in humans, dogs and rats (Hoobler, '17; Adair, '25; Hitchcock, '26; Daggs, '31, and many others). Of these liver seems to be the outstanding one. If the dietary

protein level is too high, kidney hypertrophy and some kidney damage may result. Maynard and Bender ('27, '28) believe that, even though high protein diets do produce enlargements and some degeneration of the kidney in rats, it has no ill effects upon the good health of the animal or its ability to lactate normally.

Increasing the fat or carbohydrate in the diet above a certain optimal level has little if any effect. Meigs ('22), in reviewing this subject, states that

> some authors find that an increase of fat in the food is followed both by an increase milk yield and by an increased concentration of milk fat; others get neither of these results, still others get one without the other . . . . Where moderate amounts of carbohydrate are added to or subtracted from the ration, little or no change follows in either the composition or the quantity of milk.

It is of considerable importance to have an abundance of vitamins in the lactation diet, especially the B complex. The diet must include from three to five times the amount of vitamin B complex necessary for ordinary growth (Macy, Outhouse, Long and Graham, '27). Hartwell ('24) believes this increase is particularly needed when the protein level of the lactation diet is high. There is some disagreement as to which of the B factors is essential. Sure and Walker ('31) believe that vitamin G is not so essential as vitamin B, while Husseman and Hetler ('31) state that vitamin G is more important for lactation than vitamin B, although both are needed. Evans and Burr ('28) have shown that lactating rats need vitamin E in order to prevent muscle paralysis of the suckling young. Vitamin A is also required in fairly large quantities by the lactating mother (Sherman and MacLeod, '25). Sure ('28) was unable to demonstrate the existence of any specific fat-soluble vitamin essential for lactation other than vitamins A and D.

Several investigators have sought a dietary lactagogue among the minerals. Scharrer, Schropp and Schwaibold ('33) have found that the iodine content of the feed increases

milk production in cows. Stiner ('27) also has shown this and believes that iodine may enhance milk secretion in women. Since copper has been shown to produce such good results in blood regeneration, Sure ('27) used it as a supplement to vitamin B and found that it was of no value for lactation. The feeding of other minerals in amounts larger than necessary for optimal maintenance has no effect other than changing the mineral content of the milk.

The most promising means of stimulating lactation seems to be linked in some way with proteins. In work with dogs (Daggs, '31), it was shown that liver was a very good source of the lactation stimulating substance. Wilkinson and Nelson ('31) find a lactation promoting substance in fresh liver that is destroyed at 120°C. and is not soluble in ether. Smith and Seegers ('34) have found an alcohol and water soluble, ether insoluble, substance from liver to be effective in stimulating lactation in rats. They believe the lactagogue is not a protein, but is associated with it.

Mapson ('32) believes a water extract of autolyzed liver, which he names 'physin,' to be a lactagogue. He also says it is not a protein, but is associated with proteins and is probably of a hormone nature. It is his belief that 'physin' acts by stimulating the anterior pituitary. Jones and Gersdorff ('34) criticise Mapson and others for using Glaxo casein, which they have shown to be low in cystine and suggest that "cystine should be given serious consideration as a contributing factor."

EXPERIMENTAL

Wistar strain rats were used in the following experiments. The animals were kept in a room where the temperature was controlled to 82°F. $\pm$ 1°. The humidity was kept at about 40 per cent and the room was ventilated. The stock and breeding colony was maintained on a ration of Purina dog chow, distilled water and extra vitamin A once a week in the form of Patch's cod liver oil. By means of palpations, the pregnant females were selected for experimental diets about

4 to 7 days before parturition. The pregnant rats were placed in special cages.

Rat cages. Galvanized iron trays with 4-inch high sides were made just to fit into the ordinary square wire cages. The high sides protected the young from drafts and outside disturbances. The mothers were provided with nesting material in the form of clean thin wood chips. Water was provided in Rochester type drinking tubes. The food was placed in small wide mouth glass jars supported in tin cups. The accessory food substances, such as cod liver oil, wheat germ oil, yeast, etc., were placed in glass salt cellars supported in a small galvanized iron rack attached to the front wall of the large tray.

TABLE 1

DIET	K_1	K_2	LACTATION INDEX	NUMBER OF LITTERS
Casein (15 parts)	0.0488	0.0272	760	9
Beef liver (dried)	0.0551	0.0350	901	9
Egg (dried) (no lard)	0.0511	0.0391	902	8
Egg (dried) (no lard)	0.0503	0.0363	866	6
Beef round (dried)	0.0511	0.0376	887	7
Beef kidney (dried)	0.0519	0.0374	893	13
Stock (Purina chow)	0.0485	0.0348	833	14

The females remained on the experimental diet until the young were weaned. At parturition the litters were limited to six young. The mother and young were weighed daily. The diet was replenished and the daily dietary supplements were placed in clean salt cellars after each weighing. The method used for judging lactation is described in detail in the preceding paper. Table 2 of the preceding paper illustrated the construction of the diets.

The following tables give compilations of the results of the various diets. The diets are all designated by their sources of protein, etc., the other constituents being essentially the same in all diets, unless otherwise indicated. The protein level, with the exception of a few cases was kept at a minimum so as to determine better the qualitative difference between various protein sources. Table 1 shows the qualita-

tive differences between various natural protein sources. The meats were trimmed free of fat, ground, spread in thin layers on metal trays and dried completely by means of a current of warm air. The dried meats were ground to a powder and incorporated in the diet in fifteen part protein equivalents. The egg used was in the form of ground whole hen-egg flakes. The method of plotting the growth data is illustrated in figure 1 of the preceding paper. K_1 indicates the slope of the first straight line on the growth curve, K_2 the second line, and the lactation index is the sum of the two K's disregarding the decimal. It then becomes merely a figure of relative significance only, and a means of quickly comparing the data.

TABLE 2

DIET	K_1	K_2	LACTATION INDEX	NUMBER OF LITTERS
Liver (dried)	0.0551	0.0350	901	9
Liver (dried at high temperature)	0.0456	0.0247	703	10
Egg (dried) (no lard)	0.0503	0.0363	866	6
Egg (cooked in double boiler for 3 hours) (no lard)	0.0500	0.0370	870	6
Casein + a water extract of autolyzed liver concentrated to a paste at low temperature	0.0520	0.0375	895	3
Casein + a water extract of autolyzed liver concentrated to dryness at a high temperature	0.0464	0.0183	647	2

In order to determine whether or not the effective principle was associated with the mineral constituents, the ash of egg was added to the casein diet; when this was done the $K_1 = 0.0484$, $K_2 = 0.0273$, and the index $= 757$. This was practically identical with the casein index of 760, thus showing no improvement. Mapson ('32) has shown also that liver ash was ineffective and that added manganese produced no favorable effects.

Table 2 illustrates the effect of temperature upon the active principle.

The results of the experiments cited in table 2 show that the principle is partially heat labile or liable to oxidation in the absence of water at higher temperatures. Wilkinson and

Nelson ('31) have shown that the factor in liver is destroyed when heated to 120°C.

Table 3 illustrates the reproducibility of results and the validity of the method. It also shows, as several other investigators have shown, the alcohol solubility of at least part of the principle in liver and kidney. This does not seem to hold for egg. It is probably not easily removed by alcohol from egg.

When the alcohol soluble material from liver was freed of fat with ether and taken up in water, it did not augment the

TABLE 3

DIET	K_1	K_3	LACTATION INDEX	NUMBER OF LITTERS
Alcohol extracted liver	0.0509	0.0352	861	7
Alcohol extracted liver (2 years later)	0.0520	0.0342	862	9
Alcohol extracted egg	0.0541	0.0353	894	5
Alcohol extracted egg (2 years later)	0.0534	0.0365	899	16
Alcohol extracted kidney	0.0468	0.0364	832	4
Liver (dried)	0.0551	0.0350	901	9
Egg (dried) (no lard)	0.0511	0.0391	902	8
Kidney (dried)	0.0519	0.0374	893	13
Casein (15 parts)	0.0488	0.0272	760	9
Casein + alcohol-water soluble extract of liver	0.0476	0.0289	765	6
Alcohol extracted liver + alcohol-water soluble extract of liver	0.0548	0.0346	894	12

casein diet, but did bring the alcohol extracted liver diet to a value practically as good as that of whole dried liver. Why the alcohol water extract did not augment the casein diet is difficult to understand, unless there is more than one lactation principle.

Since no effect was obtained when the alcohol-water extract was added to casein, it seemed advisable to try the various fatty constituents, although Wilkinson and Nelson ('31) had shown that the factor still remained in the residue after ether extraction of liver, and Smith and Seegers ('34) show its absence in liver fat. Table 4 shows some of these experiments.

The only hint of betterment is seen when liver fat was added to fat extracted liver and it is quite conceivable that some of the alcohol-water soluble material could be carried along as a contaminant. The unsaponifiable fraction of the fat, lecithin, extra wheat germ oil, extra cod liver oil, or extra lard definitely hindered lactation, especially in the latter half of the

TABLE 4

DIET	K_1	K_2	LACTATION INDEX	NUMBER OF LITTERS
Casein (15 parts)	0.0488	0.0272	760	9
Casein + unsaponifiable fat from alcohol-ether extract of egg	0.0480	0.0268	748	13
Casein + crude lecithin from egg	0.0464	0.0260	724	3
Alcohol extracted liver + crude liver fat (in place of lard in the diet)	0.0545	0.0336	881	9
Alcohol extracted liver	0.0520	0.0342	862	9
Alcohol extracted liver + alcohol-water soluble extract of liver	0.0548	0.0346	894	12
Casein + 20 drops/day wheat germ oil	0.0454	0.0237	691	6
Casein + 28 parts lard	0.0511	0.0216	727	5
Casein + c.l.o. in place of lard in the diet	0.0442	0.0199	641	3

TABLE 5

DIET	K_1	K_2	LACTATION INDEX	NUMBER OF LITTERS
Casein (15 parts)	0.0488	0.0272	760	9
Casein (25 parts)	0.0568	0.0360	928	11
Alcohol extracted egg	0.0534	0.0365	899	16
Alcohol extracted egg (25 parts)	0.0565	0.0358	923	5
Casein + water extract of autolyzed liver	0.0520	0.0375	895	3
Casein + water extract of autolyzed egg	0.0530	0.0321	851	3
Casein + alcohol-water extract of liver	0.0476	0.0289	765	6
Casein + alcohol-water extract of egg	0.0477	0.0272	749	6

period. Smith and Seegers ('34) also have shown that vitamin E is not the principle involved.

These experiments seemed to limit the lactation principle to the protein fraction. This also was brought out when the fifteen part protein level was increased to twenty-five in the case of casein and alcohol extracted egg, as shown in table 5.

The experiments in table 5 confirm the work of Mapson ('32) in that the lactation principle is readily extracted from

TABLE 6

DIET	K_1	K_2	LACTATION INDEX	NUMBER OF LITTERS
Casein (15 parts)	0.0488	0.0272	760	9
Casein (15 parts) + Armour peptone (2 parts)	0.0510	0.0261	771	3
Casein (15 parts) + Witte peptone (2 parts)	0.0550	0.0335	885	3
Casein (15 parts) + blood fibrin (1 part)	0.0532	0.0322	854	3
Casein (15 parts) + lactalbumen (1 part)	0.0549	0.0310	859	4
Casein (15 parts) + glycine (1 part)	0.0494	0.0243	737	3
Casein (15 parts) + cystine (1 part)	0.0586	0.0350	936	6
Casein (15 parts(+ K_2S ($\frac{1}{2}$ part)	0.0513	0.0250	718	3
Casein (12 parts) + cystine (1 part) + glycine (1 part) + glutamic acid (1 part)	0.0628	0.0360	988	3

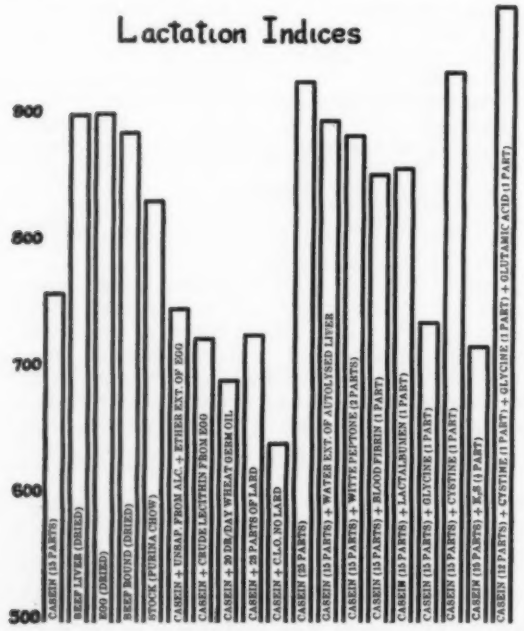
Lactation Indices
900
800
700
600
500
CASEIN (15 PARTS)
BEEF LIVER (DRIED)
EGG (DRIED)
BEEF ROUND (DRIED)
STOCK (PURINA CHOW)
CASEIN + UNSAP. FROM ALC. + ETHER EXT OF EGG
CASEIN + CRUDE LECITHIN FROM EGG
CASEIN + 20 DR. DAY WHEAT GERM OIL
CASEIN + 25 PARTS OF LARD
CASEIN + CLO. NO LARD
CASEIN (15 PARTS)
CASEIN (15 PARTS) + WATER EXT OF AUTOLYSED LIVER
CASEIN (15 PARTS) + WITTE PEPTONE (2 PARTS)
CASEIN (15 PARTS) + BLOOD FIBRIN (1 PART)
CASEIN (15 PARTS) + LACTALBUMEN (1 PART)
CASEIN (15 PARTS) + GLYCINE (1 PART)
CASEIN (15 PARTS) + CYSTINE (1 PART)
CASEIN (15 PARTS) + K_2S (1 PART)
CASEIN (12 PARTS) + CYSTINE (1 PART) + GLYCINE (1 PART) + GLUTAMIC ACID (1 PART)

Figure 1

autolyzed liver by means of water. This is also true of autolyzed egg. A larger amount can be extracted in this manner than by alcohol extraction of fresh liver or whole egg.

Since autolysis released a considerable amount of the lactagogue, it was logical to try some of the degradation products of animal proteins.

It will be seen from table 6 that Witte's peptone was better than Armour's. Since we do not know how these peptones are made, we cannot speculate as to the reason for the difference. Blood fibrin, which is the source of some peptones, makes a fair showing. Glycine alone is very poor, but cystine shows a tremendous stimulating effect; in fact, better than anything tried before, even those experiments where 25 parts of liver or egg were used. The effect was evidently not due to added sulphur, since the experiments where K_2S was used gave very poor results. When a synthetic mixture of cystine, glycine and glutamic acid was made to simulate glutathione and fed with the extremely low amount of casein protein, very striking results were obtained. Even if we consider the amino acids sources of protein, the total protein level of the diet is only 15 per cent. It seems, then, that it is not necessary to feed high protein diets in order to maintain a high level of milk production. The high protein diets probably supplied the extra cystine or glutathione constituents necessary for lactation. Figure 1 better illustrates the relative lactation values of some of the diets used.

DISCUSSION

In a short article in Science, Hammett ('34) discusses the function of glutathione. He states,

> Thus, then, it seems as if in glutathione nature has developed in one and the same chemical compound, a complex which conditions if it does not determine the course of the several basic and essential processes concerned in developmental growth. Through cysteine it accelerates cell proliferation, the first step; through glycine it accelerates that protein reconstruction which is an essential accompaniment to both cell division and cellular differentiation; and through glutamic

acid it accelerates the progress of that selective building up of the protein molecule which is the characterizing process of differentiation and its consequent organization.

It may be that cystine or glutathione stimulates the mammary gland as such or is merely passed on to the suckling young through the milk and there acts as a growth stimulant. In order to gain some light on this question, we tried to feed a water suspension of cystine directly to the suckling young of mothers on a basal casein diet. It is very difficult and unsatisfactory to feed cystine to such small beings as suckling rats, but for the few experiments successfully completed the values were $K_1 = 0.0468$, $K_2 = 0.0284$ and a lactation index of 752, slightly below that of a litter from a casein fed mother receiving no cystine. We intend to repeat the experiment on larger animals.

It is definitely known that casein is relatively low in cystine and it may be surmised that the added cystine is merely making a more complete protein of casein. But liver and egg are both considered sources of good complete proteins and even when fed at practically two times the level they did not give as good results as the casein + cystine diet.

The reason why liver is so good for lactation may well be that it contains more glutathione than any other animal tissue. No fact developed in this study stands in the way of this explanation. There is little if any glutathione in fresh hen's egg, but the cystine content is relatively high. Since hot alcohol can 'peptize' proteins, it is probable that the alcohol-water extracts contain cystine-yielding protein products and thus stimulate lactation because of their cystine content. Water soluble proteins from vegetables have been found to contain fair amounts of cystine.

The criticism may arise as to the toxicity of cystine. The percentage is hardly high enough to produce any ill effects. Longwell, Hill and Lewis ('32) have shown that cystine is not toxic when fed with vitamin B complex, and our diets do contain an abundance of B complex.

It is our belief that the lactation augmenting effect of high protein diets, liver extracts, etc., is due to the extra amount of cystine or glutathione contained in the diet and that glutathione particularly acts in some way as a mammary stimulant.

SUMMARY

By using the method described in the preceding paper, lactation studies were carried out on rats using various dietary principles. The food materials tested were either fed in place of the casein of the basal diet or as added daily supplements.

The lactation promoting factor was found to be present in liver, egg, round, kidney, alcohol-water extract of liver, diets of higher protein level (egg casein), water extract of autolyzed liver or egg, Witte's peptone, blood fibrin, lactalbumen, cystine and a combination of 1 part cystine, 1 part glycine and 1 part glutamic acid fed with only 12 parts of casein and the other constituents of the basal diet. This last mixture which simulates glutathione gave the best results, although cystine alone as supplement showed a remarkable effect.

The factor was not present in the unsaponifiable fat of egg, in lecithin, extra wheat germ oil, extra cod liver oil, extra lard, Armour's peptone, glycine or K_2S.

The authors believe that cystine or glutathione acts in some was as a mammary stimulant.

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THE EFFECT OF GRAPE AS COMPARED WITH OTHER FRUIT JUICES ON URINARY ACIDITY AND THE EXCRETION OF ORGANIC ACIDS [1]

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SIX FIGURES

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INTRODUCTION

Effect of grape juice on urinary acidity. Numerous well-known experiments have clearly established the fact that the ingestion of large quantities of most fruits and vegetables leads, first, to an appreciable decrease in urinary acidity and, second, to an increased output of organic acids. The increased output of organic acids has usually been attributed to the elimination of a certain fraction of the fruit acids which has escaped oxidation in the tissues, although the presence of these acids in the urine has never been proved. Certain fruits, however, notably prunes, plums, and cranberries, have been observed to have an opposite effect, that is, to increase rather than decrease the total acidity of the urine (Blatherwick and Long, '23) and it has been further observed (Slattery, '25) that the ingestion of from 600 to 750 cc. of apple juice (sweet cider) may reduce, rather than increase, the organic acid content of the urine.

That grape juice may also induce a reaction different from that of other fruits is indicated by the conflicting results obtained in certain recent investigations. Pickens and Hetler

[1] Part of a dissertation submitted to the faculty of the Division of the Biological Sciences of The University of Chicago in candidacy for the degree of doctor of philosophy.

('28) have found that as much as 1 quart of grape juice daily either had no effect upon, or resulted in a slight lowering of the pH of the urine, while Saywell ('32) and Pratt and Swartout ('33 a) have observed that similar quantities of grape juice produced a marked rise in the pH of the urine of their subjects. Pickens and Hetler were, however, working with commercial Concord juice, while the other investigators have used the fresh juice of several varieties of grapes, including the Concord. The difference between his results and those of Pickens and Hetler, Saywell explains on the basis of the probable difference in alkalinity of the fruit juices used, the commercially bottled juice being considerably less alkaline than the fresh, because of the precipitation of tartrates during storage. It is also very probable that different varieties of grapes differ markedly in alkalinity of ash, since the alkalinity of fresh Concord grape juice is reported by Hartmann and Tolman ('18) to be equivalent to 48.6 cc. 0.1 N alkali per 100 cc. of juice, while Saywell reports the alkalinity of the ash of his mixture of fresh Malaga, Muscat and Petit Sirah juices to be equivalent to 74.2 cc. 0.1 N alkali per 100 gm. of juice. In this connection it should be noted that Pratt and Swartout ('33) have observed that Rochelle salts were considerably more effective in reducing the acidity of the urine than was potassium bitartrate—a result which is consistent with the difference in alkalinity of the two compounds.

Relation of organic acid excretion to acid-base balance. It has recently been suggested (Fasold, '30; Schuck, '34 a) that the excretion of organic acids in normal urine may be an important factor in the maintenance of the acid-base balance of the blood of healthy persons. Fasold has observed that the addition of sodium bicarbonate to an already alkaline (vegetable) diet greatly increases the output of organic acids, while the addition of hydrochloric acid to an acid (meat) diet greatly reduces the total organic acid excretion. He has also found ('31) that the administration of sodium or potassium salts of organic acids, such as citric or lactic acids, has the same effect upon the excretion of organic acids as has sodium bicarbonate,

while the free organic acids have the same effect as mineral acids, that is, they bring about a reduction in the total organic acids eliminated. The output of any given organic acid, for example, citric or lactic acid, when given by mouth, is sometimes increased, but may actually be reduced in the urine, especially in children. These results have recently been confirmed and extended by Schuck ('34 b) who reports that total organic acid excretion was decreased by the addition of citric acid to a constant, slightly acid basal diet, but was doubled or even tripled by the addition of chemically equivalent amounts of sodium citrate. Citric acid excretion was decreased in some cases and increased in others as the result of the citric acid ingestion, while the sodium citrate brought about a marked increase in every case. Additions of sodium bicarbonate to the basal diet also brought about small increases in the total organic acid excretion and large increases in the citric acid.

Thus, when alkalies are fed the organic acids in general, and citric acid in particular, appear to be important factors in excreting the excess. However, the output of no one acid, even citric acid (Schuck, '34 a), is sufficiently increased to account for the total increase in organic acid excretion. According to Fasold, therefore, "it is possible that there is a group of acids which . . . . through the summation of their small increase in administration of alkali, effect a greatly increased output." Of the organic acids which may function as alkali excretors Fasold believes citric acid to be the most important. Others acids which may play a minor role are lactic and hippuric.

It is very probable, therefore, that the increased organic acid excretion which usually accompanies a fruit diet does not simply represent the excretion of unoxidized portions of the fruit acids, but is one of the mechanisms involved in the adjustment of the acid-base balance. As further evidence for this view, McLaughlin and Blunt's observation ('23) of an increased excretion of uric acid after a fruit meal may be cited.

EXPERIMENTAL

The following investigation was undertaken for the purpose of studying the effect of grape juice as compared with the juice of other fruits and vegetables on the acidity and total organic acid content of the urine. A second aim was to determine, if possible, the identity of the organic acids excreted on the fruit juice diets. The investigation was divided into three separate parts which will be described as experiments I, II and III.

Experiment I

Method: General procedure and diet. The first experiment was intended to be preliminary only, but the results are of sufficient interest to warrant their inclusion here. Five normal young women acted as subjects and two different fruit or vegetable juices were tested on each subject. The experiment was divided into four periods of 3 days each, as follows: period 1, basal diet; period 2, basal diet plus 1 pint of a specified fruit juice; period 3, basal diet; period 4, basal diet plus 1 pint of a second fruit juice. One preliminary day for adjustment to the fruit juice diet was usually allowed between the basal and the fruit juice periods. The basal diet was nearly neutral in reaction and the fluid intake was kept constant at about 2 liters.

The fruit and vegetable juices used were commercially bottled grape juice, both Concord and Catawba; freshly pressed juice from Thompson seedless grapes; freshly pressed orange, lemon, grapefruit, and pineapple juices; commercially bottled loganberry juice, canned sauerkraut juice; and canned tomato pulp.

Chemical analyses. The following determinations were made on 24-hour specimens of urine, collected and preserved under toluene: pH, colorimetric; titratable acidity, according to Folin ('03); and organic acids according to Van Slyke and Palmer ('20).

Results. A summary of the results of this experiment, together with the estimated alkalinity of the ash of certain of the fruit juices used, is given in table 1. All of the juices tested

were effective in decreasing the acidity of the urine, although all were not equally so. In general, the rise in the pH of the urine appeared to parallel the increase in alkalinity of the ash of the fruit juice used. Of the three varieties of grape juice tested, the fresh Thompson seedless juice was most effective in reducing urinary acidity, while the bottled Concord juice

TABLE 1

Average increase in urinary pH and total organic acid excretion of five normal women on diets containing 1 pint of a specified fruit or vegetable juice, together with the total alkalinity of the ash of some of the fruit juices used[1]

FRUIT JUICE	URINE		FRUIT JUICES
	Average increase in pH	Excess organic acid	Excess alkalinity of ash per 100 gm. juice[2]
		per cent of intake	*cc. N*
Lemon	0.4	8.1	4.55
Grape, Concord (bottled)	0.5	12.5	1.94
Grape, Catawba (bottled)	0.6	7.0	....
Grapefruit	0.6	5.9	4.08
Grape, Thompson seedless (fresh)	0.7	7.3	....
Loganberry	0.7	1.9	....
Sauerkraut	0.7	7.5	....
Orange	0.8	11.8	5.70
Pineapple	0.8	10.7	7.20
Tomato	1.0	11.4	7.19

[1] Figures for Catawba and Thompson seedless grapes, and for loganberry and sauerkraut juices are omitted in the table because average figures for these juices were not found.

[2] Estimated from Sherman ('27) and Sherman and Gettler ('12).

was least so. It seems highly probable that this result reflects a difference in the alkalinity of the ash of the three varieties of grapes. A further, very interesting and suggestive result of this experiment was that, with the exception of the Concord grape juice, the greatest increase in organic acid excretion occurred on the diets containing the most highly alkaline fruit juices.

Experiment II

The second experiment was planned to make a further study of the effects of grape as compared with orange and apple juices upon urinary acidity and also upon the calcium and phosphorus retention of adults.[2]

Orange juice was selected because of the widespread popularity of this fruit juice and because of the reports of other investigators as to its effectiveness in reducing urinary acidity (Blatherwick and Long, '22; Chaney and Blunt, '25) and in promoting calcium and phosphorus retention in children (Chaney and Blunt, '25). The apple juice was chosen because it seemed desirable to investigate further the decreased organic acid excretion found by Slattery ('25).

Method: General procedure. Since calcium and phosphorus retentions were to be determined, a complete metabolism study was carried out on each subject. A low-calcium weighed diet was taken, and both urine and feces, together with aliquot portions of the food, were collected and preserved according to the accepted technic for such studies.

Three normal women served as subjects. Since it seemed desirable to study the effects of all three juices in each of the three subjects, the experiment was divided into six periods, as follows: period 1, basal diet; period 2, basal diet plus 525 gm. bottled apple juice (commercial sweet cider); period 3, basal diet; period 4, basal diet plus 500 gm. freshly pressed orange juice; period 5, basal diet; period 6, basal diet plus 500 gm. bottled Concord grape juice. A preliminary period of 3 days on the basal diet, without collection of urine or feces, preceded period 1. The other periods were separated by one preliminary day in which the fruit juice was fed, but urine collections were not made.

Diet. Computation of the acid-base content of the various diets showed that the basal diet was moderately acid (table 2). The apple and grape juice diets were also slightly acid, but less so than the basal diet, while the orange juice diet was

[2] The data on calcium and phosphorus retention will be reported in a later paper.

distinctly alkaline. Using Hartmann and Hillig's ('30) figures for the tartaric and citric acid content of grapes and oranges, respectively, and the figures furnished by Slattery ('25) for the malic acid content of apple juice, the intake of these acids in the quantities of fruit juice ingested was computed to be 2.1 to 3.9 gm. malic acid in the apple juice, 4.3 gm. citric acid in the orange juice, and 3.8 gm. tartaric acid in the grape juice.

Chemical analyses. The following determinations were made on the 24-hour specimens of urine: pH, colorimetric, according to the method of Hastings, Sendroy and Robson ('25) with the use of the Hastings bicolorimeter; titratable acidity, Folin;

TABLE 2

Acid-base content of diets used in experiment II[1]

DIET	PERIOD	EXCESS IN RESIDUE	
		Acid	Base
		cc. N	*cc. N*
Basal	1, 3, 5	19.5–23.4	
Basal, plus 525 gm. apple juice	2	5.8–10.1	
Basal, plus 500 gm. orange juice	4		4.7–8.5
Basal, plus 500 gm. grape juice	6	9.8–13.4	

[1] Estimated from Sherman ('27).

total organic acids, Van Slyke and Palmer ('20); and ammonia, Folin and Bell ('17).

Results. In this experiment apple juice proved to be strongly laxative for two of the subjects, but not at all so for the third. The other two fruit juices were mildly laxative for one subject, but not so for the others. The catharsis appears to have had little consistent effect upon the acidity of the urine, however.

The difference in the effects of the three fruit juices is clearly shown in figures 1 and 2. Figure 1 indicates that, in general, the pH of the urine was raised when approximately ½ liter of orange juice was ingested, but remained the same when similar quantities of apple or grape juice were taken. Figure 2 shows that the total acid excretion (titratable acid

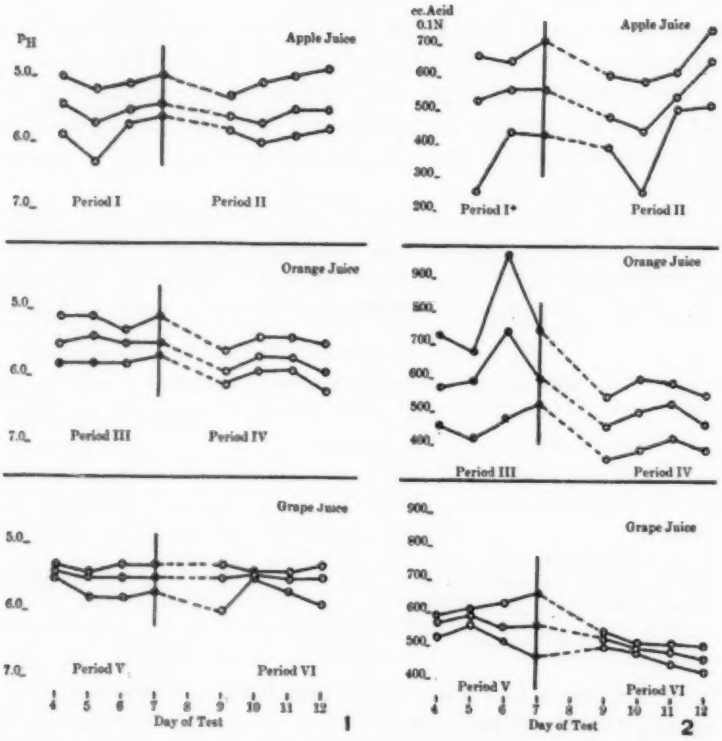

Fig. 1 Maximum, minimum and average pH of urine of three subjects on a basal diet and on the same diet supplemented with 500 gm. of a specified fruit juice (525 gm. in the case of apple juice). The heavy vertical line indicates the end of the basal diet. The dotted line indicates preliminary period on fruit juice diet.

Fig. 2 Maximum, minimum and average excretion of total acid (titratable acid plus ammonia) by three subjects on a basal diet and on the same diet supplemented with 500 gm. of a specified fruit juice (525 gm. in the case of apple juice). Heavy vertical line indicates end of basal diet. Dotted line indicates preliminary period on fruit juice diet. * Ammonia not determined on first test day.

plus ammonia) was reduced on both the grape and the orange juice diets, but that the reduction was somewhat less marked when the grape juice was fed. On the apple juice diet, on the contrary, the total acid excretion tended to increase rather than decrease. A comparison of figures 1 and 2 shows, rather surprisingly, that on the grape juice diet the reduction in total acid excretion was considerably greater than would be indicated by the very slight change in pH.

Organic acids. The excess of total organic acids excreted during the fruit juice periods, expressed in terms of per cent of intake, was greatest during period 4, when orange juice was fed. On the apple juice diet the organic acid excretion was actually reduced in two subjects, although an increase amounting to 6.4 per cent of the intake was observed in the third. In figure 3 are plotted the maximum, minimum, and average excretion of total organic acids in all six experimental periods. A comparison of figures 2 and 3 indicates that the organic acid excretion tended to be the reverse of the total acid excretion; that is, as the total acid output was reduced the organic acid output tended to rise. When there was little change in the total acid excretion, as on the apple juice diets in this experiment, the organic acid output tended to remain practically unchanged.

The CO_2-combining power of the plasma. Fitz and Van Slyke ('17) have found that the total acid excretion (titratable acid plus ammonia) bears a fairly close relationship to the CO_2-combining power of the blood, and that the latter may be computed roughly, with an error of from 3.2 to 7 volumes per cent when the following formula is used:

$$\text{Plasma } CO_2 \text{ capacity} = 80 - \sqrt{D/W}\sqrt{C}$$

where 80 is the maximum CO_2-combining power of the plasma, D the rate of excretion of 0.1 N acid plus ammonia per 24 hours, W the weight of the individual and C the cc. of 0.1 N acid plus ammonia per liter of urine. Recently, Bischoff, Sansum, Long and Dewar ('34) have reported that they could find no correlation between the plasma bicarbonate and either

the pH or the titratable acidity of the urine and, further, that any changes which did occur in the plasma bicarbonate as a result of feeding substances having either an acid or an alkaline residue were well within the range of error reported by Fitz and Van Slyke, and also within the range of variation

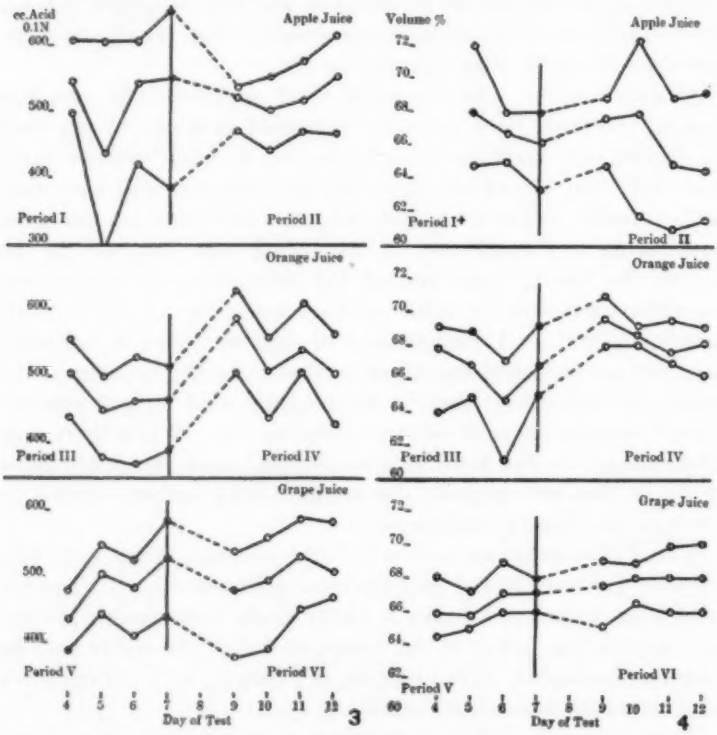

Fig. 3 Maximum, minimum and average excretion of total organic acids by three subjects on a basal diet and on the same diet supplemented with 500 gm. of a specified fruit juice (525 gm. in the case of apple juice). Heavy vertical line indicates end of basal diet. Dotted line indicates preliminary period on fruit juice diet.

Fig. 4 Maximum, minimum and average CO_2 binding power of the plasma of three subjects on a basal diet and on the same diet supplemented with 500 gm. of a specified fruit juice (525 gm. in the case of apple juice). Heavy vertical line indicates end of basal diet. Dotted line indicates preliminary period on fruit juice. + Ammonia not determined on first test day.

found in the fasting blood (taken before breakfast) of subjects on an ordinary diet. It has, nevertheless, seemed worth while to compute the values for the CO_2-combining power of the plasma for the subjects of this experiment according to the Fitz and Van Slyke formula. The results of these computations are presented in figure 4, which shows an apparent tendency of the blood to become somewhat more acid on the apple juice diet and somewhat more alkaline on both the orange and the grape juice diets. It should be observed, however, that all of the values found in this experiment are within the normal range reported by Fitz and Van Slyke, and also, for the most part, within the range of error reported for the computation. It is therefore quite possible that no significant changes occurred in the blood, although the fact that the different juices appear to have caused different reactions seems of interest.

Experiment III

Method: General procedure. Experiment III was essentially a repetition of experiment II, except that the quantity of fruit juice taken was increased to 1000 gm. daily. Two fruit juices only, orange and grape (commercially bottled Concord), were included and but two subjects were used in the study of each fruit juice.

Diet. The basal diet used in this experiment had a potential acidity of 4.9 to 5.3 cc. of normal acid daily. Both of the fruit juice diets were potentially alkaline, the grape juice diet having a potential alkalinity of 13.0 to 14.1 cc. of normal alkali per day, while the orange juice diet contained an excess of 50.2 to 51.2 cc. of normal alkali daily. The tartaric acid intake in the grape juice diets, estimated as in experiment II, was probably in the neighborhood of 7.6 gm. daily. Since Hartmann and Hillig ('30) have recently reported that grapes also contain an average of 0.023 per cent of citric acid, the intake of citric acid on the grape juice diet probably amounted to about 0.23 gm. daily. The citric acid in the orange juice, as determined by Schuck ('34 a) amounted to 8.51 gm. daily.

Chemical analyses. pH, titratable acidity, and total organic acids were determined on the 24-hour specimens of urine as in experiment I. Ammonia determinations were not made, but instead the uric acid and citric acid output was determined daily, the former by the Folin-Shaffer method (described by Mathews, '30), and the latter by the method described by McClure ('22).[3]

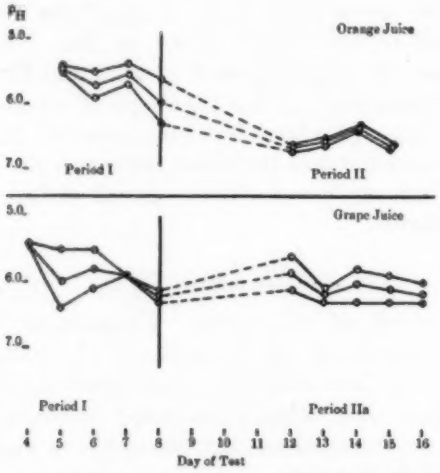

Fig. 5 Maximum, minimum and average pH of urine of two subjects on a basal diet and on the same diet supplemented with 1000 gm. of a specified fruit juice. Heavy vertical line indicates end of basal diet. Dotted line indicates preliminary period on fruit juice diet.

Results. No laxative effects of either of the fruit juices used were felt by any of the subjects in this experiment.

Urinary acidity and total organic acids. The results of experiment III are presented in figures 5 and 6. Figure 5 shows that the larger quantity of orange juice brought about a very distinct lowering of the acidity of the urine, the effect of 1000 gm. of orange juice being much more marked than that of a similar quantity of grape juice. A comparison of

[3] The citric acid determinations were made by Miss Cecilia Shuck as a part of a further study of the metabolism of this acid ('34 a).

figures 1 and 5 also shows that the larger quantities of juice were decidedly more alkalinizing than the smaller quantities used in experiment II.

Figure 6 shows that in this experiment the increase in the output of total organic acids was quite similar on the two

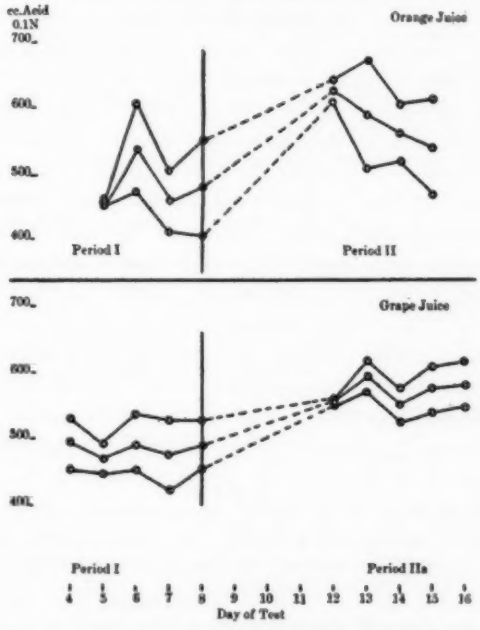

Fig. 6 Maximum, minimum and average excretion of total organic acids by two subjects on a basal diet and on the same diet supplemented with 1000 gm. of a specified fruit juice. Heavy vertical line indicates end of basal diet. Dotted line indicates preliminary period on fruit juice diet.

juices, the increase again being greater than that which occurred in experiment II (fig. 3).

Uric and citric acids. Table 3 shows that the output of both uric and citric acids was increased in all subjects on both the grape and orange juice diets. The increased uric acid output, while not large, was sufficient to account for 2.3 to 12.8 per cent of the average increase in total organic acids during the

fruit juice periods. On the orange juice diet the increase in citric acid output amounted to an average of 0.234 and 0.457 gm. per day, or 2.8 to 5.4 per cent of the intake. On the grape

TABLE 3

Average total organic, uric and citric acid output of four normal women on a basal diet and on the same diet supplemented with 100 gm. of a specified fruit juice

DIET	SUBJECT	ACID OUTPUT PER 24 HOURS		
		Total organic	Uric	Citric
Grape juice				
		cc. 0.1 N	*cc. 0.1 N*	*cc. 0.1 N*
Basal	C	525	0.394	0.871
	H	447	0.363	0.634
Basal plus 1000 gm. grape juice	C	596	0.470	1.019
	H	549	0.382	0.909
Orange juice				
Basal	R	512	0.341	0.658
	K	442	0.441	0.452
Basal plus 1000 gm. orange juice	R	625	0.422	1.115
	K	537	0.479	0.686

TABLE 4

Comparison of the sum of the excess uric plus citric acids excreted on diets containing 1000 gm. of a specified fruit juice with the excess of total organic acids excreted on the same diet

FRUIT JUICE	SUBJECT	EXCESS ACID						
		Total organic	Uric	Citric	Uric plus citric		Undetermined	
		cc. 0.1N	*cc. 0.1N*	*cc. 0.1N*	*cc. 0.1N*	*per cent*	*cc. 0.1N*	*per cent*
Orange	K	94.0	4.5	36.5	41.0	44	53.0	56
Orange	R	113.0	9.6	71.4	81.0	72	32.0	28
Grape	C	70.2	9.0	23.1	32.1	36	48.1	64
Grape	H	100.4	2.3	41.7	44.0	44	56.4	56

juice diet the increase in citric acid amounted to the surprisingly high average of 0.148 and 0.275 gm. per day, an increase of 64 per cent of the intake in one subject, and more than 100 per cent in the other. Table 4 shows that the sum of the

excess uric plus citric acids in the urine during the fruit juice periods represents 44 and 72 per cent of the excess organic acid on the orange juice diet, and 36 and 44 per cent of the excess acid output on the grape juice diet. This leaves approximately one-quarter to one-half of the excess organic acid unaccounted for on the orange juice, and about one-half to two-thirds unaccounted for on the grape juice diets.

It is regrettable that determinations of tartaric acid output could not have been made in this study. However, the work of other investigators throws some light on this point. Pratt and Swartout ('33 b) were unable to find tartaric acid in the urine of their subjects after the ingestion of 1000 gm. grape juice, although they say that they "could not devise a practical procedure that would detect tartrates in a solution more dilute than one part in two thousand." Finkle ('33) has observed that 85 to 98.5 per cent of pure neutral sodium tartrate injected intramuscularly appeared in the urine within a few hours, but both Finkle and Underhill and his associates ('31) report that when from 1 to 10 gm. of either tartaric acid or its salts are taken by mouth a maximum of 20 per cent of the quantity ingested appears in the urine. No tartaric acid has been found in the feces in the investigations quoted, but the decomposition of the tartrates by human intestinal contents and by the common fecal bacteria has been observed by Pratt and Swartout ('33 b) and by Underhill and his associates ('31). It is concluded by the latter investigators that a small portion only of the tartrate taken by mouth is absorbed by man and that the human body has no power of oxidation of tartaric acid.

In view of the above facts, it is possible, though it hardly seems probable, that the entire amount of undetermined excess acid in the urine on the grape juice diet was tartaric. If such had been the case, the total excretion of tartaric acid would have amounted to something less than 5 per cent of the intake. In any event, it is not possible to explain the failure of grape juice to lower the pH of the urine as efficiently as does orange juice on the basis of the excretion of large quantities of tartaric acid.

SUMMARY

The effect of various fruit and vegetable juices, especially of bottled apple juice, fresh orange juice, and bottled Concord grape juice, upon the urinary acidity and total organic acid output of normal adult women has been compared, with the following results:

1. The ingestion of orange juice has produced a more marked effect upon the alkalinity of the urine (as measured by an increase in pH and a reduction in titratable acidity and ammonia) than has the ingestion of either grape or apple juice.

2. The effect of bottled Concord grape juice has been variable, although usually in the direction of an alkalinization of the urine.

3. The effect of apple juice has also been variable, with a tendency toward an increased acidity of the urine.

4. Both grape and orange juices tend to produce an increase in the CO_2-combining power of the blood, as calculated by the Fitz and Van Slyke formula. Apple juice, on the contrary, tends to produce a decrease in CO_2-combining power.

5. The ingestion of both grape and orange juices produces an increased excretion of organic acids. The ingestion of apple juice, however, is often accompanied by a decreased organic acid output.

6. The increase in organic acid output observed on both the orange and grape juice diets is in part due to an increased excretion of uric plus citric acids. The increase in organic acid output is not entirely due, therefore, to the excretion of unoxidized remnants of individual fruit acids.

7. The data on organic acid excretion obtained in this investigation are believed to support the suggestion that the organic acids play a significant role in the maintenance of acid-base balance in the body.

Acknowledgment is gratefully made to Dr. Katharine Blunt for the suggestion of this problem; to Dr. Lydia Roberts and other members of the Department of Home Economics and Household Administration for their helpful interest and advice during the progress of the work; to Mrs. Vida Wentz for technical assistance in the determination of ammonia in the second experiment; and to the students who willingly served as subjects in the various experiments.

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EXPERIMENTAL STUDIES ON THE HYPOPHYSIS CEREBRI

IV. THE EFFECT OF VITAMIN E DEFICIENCY IN THE FEMALE ALBINO RAT

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INTRODUCTION

Vitamin E deficiency seems to operate differently from general inanition and various other combined inanition factors in bringing about sterility, especially in the female. Since it had been proved that the hypophysis was closely related to reproduction (Smith, '26; Smith and Engle, '27; Ascheim and Zondek, '27; Evans and Simpson, '28), investigators sought to determine in what manner the hypophysis affected or was affected by this type of sterility.

Evans, Burr and Althausen ('27) found on feeding various tissues to animals deficient in vitamin E that the hypophysis was slightly richer in that vitamin than any other gland. Vérzar ('29) showed that an abundance of vitamin E has a great stimulating action on the sexual cycle. Preparations containing vitamin E, administered either by mouth or subcutaneously, induced precocious sexual maturity in young female rats, acting similar to anterior lobe hormone. Vitamin E also required the presence of the ovaries for its activity, indicating that it is not directly a building stone of the ovarian hormone, but rather a stimulating or regulating substance for the ovary, as is also the secretion of the anterior lobe of the hypophysis. Vérzar suggested that vitamin E may even be necessary for the building up of the latter.

The earliest anatomical report regarding the effect of vitamin E deficiency on the hypophysis was made by Van Wagenen ('25), who found that in the male rat the hypophysis resembles that of a gonadectomized animal Similar findings for the male have been reported by Nelson ('32, '33) and Mason ('33).

The female rat hypophysis is apparently not affected by a deficiency in vitamin E, according to Stein ('31, a preliminary report), Nelson ('32, '33) and Mason ('33). Only Biceglie ('28) claims to have found a scarcity of acidophil and basophil cells in E-deficient female rats. He attributes these changes to profound degeneration of the ovaries, which also is an observation that none of the other authors have reported. In order to check the above findings more carefully, data derived from ninety-one female albino rats have been worked up statistically and summarized in this paper.

MATERIAL AND METHODS

A. Material

The hypophyses used in this investigation were derived from ninety-one female albino rats (Mus norvegicus albinus) of standard vigorous stock carefully reared and kept from birth to time of autopsy. These animals were used by Dr. J. A. Urner in his studies of "Changes in the uterus and mammary glands of white rats deficient in vitamin E." The following account taken from his outline indicates how the animals were reared.

The low vitamin E diet used in this investigation was developed and furnished by Dr. G. O. Burr, and consisted of casein 60, sucrose 90, salts 9 and lard 40. In addition each animal was individually fed 0.6 gram of powdered yeast and three drops of cod liver oil. This diet was selected because it was found by Evans and Burr ('25) that similar diets, high in sugar, fulfilled all the requirements necessary for growth, but gave no first litter fertility.

The animals were mated on or about the seventy-fifth day of life. The finding of sperm in a vaginal smear, taken twelve

hours after the female was put into the male's cage, was considered as evidence that fertilization had taken place and gestation was considered as having begun. When a positive breeding was diagnosed by finding of sperm, a vaginal smear was taken on the fourteenth day for the red blood cell sign. When this was found, the animal was isolated and weighed daily up to the twenty-fifth day, to detect resorbing or normal pregnancy.

The careful rearing and control of this group of pregnant animals, on a low vitamin E diet, from birth through their first pregnancy permitted establishment of a normal for these animals deprived of vitamin E. They were quite normal in appearance in all respects save that the young were absorbed.

After the entire group of animals were carried through this 'sterility' observation on the low vitamin E diet, the animals were devided into two groups. One remained on the diet used throughout the early work, and the other received a 'curative' dose of 0.4 gram of wheat germ daily. Approximately forty animals remained in each group

The second breeding was carried out in the same manner as the first and when a positive breeding was diagnosed, the animal was scheduled for killing upon some particular day of her pregnancy. The cured animals were given their cured ration, beginning with the date of finding sperm in the vagina.

No attempt was made to select animals for one group or the other, and no attention was paid to the division of sister litter mates.

Beginning with the seventh day of gestation, animals of the 'low E' group and 'cured' controls were killed on each successive day, up to and including the twenty-second day.

B. Methods

The animals were killed by being placed in a closed jar containing chloroform.

The gross weight, body length and tail length were recorded. The material was fixed in 10 per cent neutral formalin.

The sections were cut at 5 μ. A volumetric study of the three lobes, a differential cell count and a histologic survey of the anterior lobe were made.

For the volumetric procedure the method followed was that of Hammer ('14). Every tenth section was mounted and

stained with haematoxylin and eosin. The image of each section, magnified 33 diameters, was out-lined on paper of uniform thickness, cut out, and the paper weighed on a chemical balance, from which the percentage of each lobe was calculated.

For the histological study and differential cell count, about five sections at well-distributed intervals were taken, stained with a modified Mallory's triple connective tissue stain so that the nuclei stood out clearly, and made the cell count more reliable.

Three types of cells were distinguished similar to most classifications, namely, eosinophils, basophils and neutrophils.

About 8000 cells were counted in each gland.

No study of pars tuberalis was made for it had been left attached to the brain during autopsy procedure.

For further details regarding technical methods and for statistical formulae, the reader is referred to previous reports of the author's (Stein, '33 a, '33 b).

I want to take this opportunity to thank my advisers, Dr. A. T. Rasmussen, and Dr. C. M. Jackson, for the valuable information they supplied during the course of the work, and Dr. J. C. Urner for permitting me to use the material which made this study possible.

RESULTS

A. Age, body weight and body length

A difference of 17 days exists between the average age of the two groups of animals. This is a possible significant difference (table 1), but probably does not have any effect on the results, for organ weights are much more closely related to body length or body weight (Freudenberger, '32; Donaldson, '24).

No significant difference is found in body weight (table 2) or body length (table 3).

B. Weight of the whole hypophysis

Since pregnancy does not cause any change in the whole weight of the hypophysis in the rat (Stein, '33 b, '34), any change that had occurred in either of these two groups would

TABLE 1

Statistical constants for age of animal, days

ANIMALS	NO.	M ± S.E.m[1]	Diff. ± S.E.Diff[1]	Diff. S.E.Diff.[1]	P[1]	C.V.[1]
E-deficient	19	136 ± 5.39				17
E-cured	19	153 ± 6.45	17 ± 8.41	2.02	4.3	18.3

[1] M = mean; S.E.m = standard error of mean; Diff. = difference between the mean of the control and the mean of the test; S.E.Diff. = difference divided by the standard error of the difference; P = number of chances in 100 that the differences may be due to errors in random sampling; C.V. = coefficient of variation.

TABLE 2

Statistical constants for body weight, grams

ANIMALS	NO.	M ± S.E.m	Diff. ± S.E.Diff.	Diff. S.E.Diff.	P	C.V.
E-deficient	19	229.6 ± 5.40				10.2
E-cured	19	241 ± 7.03	11.4± 8.87	1.29	20.0	12.7

TABLE 3

Statistical constants for body length, centimeters

ANIMALS	NO.	M ± S.E.m	Diff. ± S.E.Diff.	Diff. S.E.Diff.	P	C.V.
E-deficient	19	20.3 ± 0.49				10.5
E-cured	19	20.3 ± 0.37	0 ± 0.61	0	0	7.8

TABLE 4

Statistical constants for weight of whole hypophysis, milligrams

ANIMALS	NO.	M ± S.E.m	Diff. ± S.E.Diff.	Diff. S.E.Diff.	P	C.V.
E-deficient	19	12.41 ± 0.48				16.3
E-cured	19	12.8 ± 0.67	0.39 ± 0.82	0.47	63.0	22.8

undoubtedly be due to the deficiency in vitamin E. However, no significant difference was found in the average weight of the whole gland of these animals (table 4).

TABLE 5

Statistical constants for absolute and relative weights of the individual lobes

ANIMALS	M ± S.E.m	Diff. ± S.E.Diff.	Diff. / S.E.Diff.	P	C.V.	M ± S.E.m	Diff. ± S.E.Diff.	Diff. / S.E.Diff.	P
	Pars anterior (absolute weight)					Pars anterior (relative weight)			
E-deficient	11.15 ± 1.08				9.6	87.77 ± 0.97			
E-cured	11.21 ± 0.64	0.06 ± 1.26	0.05	96	24.7	87.39 ± 0.57	0.38 ± 1.12	0.34	74
	Pars intermedia (absolute weight)					Pars intermedia (relative weight)			
E-deficient	0.57 ± 0.03				22.0	4.61 ± 0.21			
E-cured	0.60 ± 0.04	0.03 ± 0.05	0.64	53	26.6	4.82 ± 0.24	0.21 ±0.32	0.66	51
	Pars posterior (absolute weight)					Pars posterior (relative weight)			
E-deficient	0.93 ± 0.04				18.7	7.60 ± 0.29			
E-cured	0.96 ± 0.05	0.03 ± 0.07	0.45	65	24.0	7.78 ± 0.40	0.18 ± 0.50	0.36	72

C. Absolute and relative volume of the lobes

It is unnecessary to quote the individual figures listed in table 5 to show the striking similarity which exists between the absolute and relative weights of the various lobes of the two groups. Certainly, there is no evidence that vitamin E affects any of the individual lobes. Furthermore, on comparing the relative weights of the various lobes of these groups with those of normal non-pregnant or pregnant animals (Stein, '33 b, '34), no significant difference is found.

D. Relative number of different types of cells in the anterior lobe

A possible significant difference may be present in the differential cell count (table 6). There are about 2.8 per cent more chromophobes in the cured group, with a corresponding in-

TABLE 6

Statistical constants of percentages for differential count of cells in anterior lobe

ANIMALS	M ± S.E.m	Diff. ± S.E.Diff.	Diff. S.E.Diff.	P
		Chromophobes		
E-deficient	80.77 ± 1.00			
E-cured	83.56 ± 0.73	2.79 ± 1.24	2.25	2.4
		Acidophils		
E-deficient	17.60 ± 1.01			
E-cured	14.72± 0.73	— 2.88 ± 1.25	2.31	2.1
		Basophils		
E-deficient	1.63 ± 0.10			
E-cured	1.72 ± 0.07	0.09 ± 0.13	0.71	48

crease of about 2.9 per cent of acidophils in the deficient group, the percentage of basophils having remained equal. Since the significance of the difference between the means was tested by the statistical principle that a difference between two means is possibly significant when it is two times its standard error, and probably significant when it is three times its standard error, the differences noted above are probably not significant, for both of them are only slightly more than two times their standard error (table 6). As was mentioned, the per-

centage of basophils showed no significant difference (1.63 per cent for the deficient group and 1.72 per cent for the cured group). Since it is the basophilic cell which alters as a result of a deficiency of vitamin E in the male, the similarity of the above two figures indicated that no such change occurs in the female.

HISTOLOGY

No sign of the typical castration cell could be found in any of the glands, either of the deficient or cured group, such as were found in the vitamin E-deficient male rat hypophyses. This finding for the female is in agreement with that of Nelson ('32) and Mason ('33).

CONCLUSIONS

1. No significant differences were found in the weight of the whole hypophysis or in the absolute and relative volume of the lobes in nineteen female white rats made sterile by a deficiency of vitamin E, as compared with the lobes of the hypophysis from nineteen pregnant white rats cured of vitamin E deficiency.

2. A differential count of the cells in the anterior lobe shows that there is probably no significant difference in the percentage of the cured and non-cured. This is particularly true for the basophilic cells.

3. No evidence of any histological change could be found resulting from the vitamin E deficiency such as has been claimed for the male animal.

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THE VITAMIN E CONTENT OF EGGS AS RELATED TO THE DIET OF THE HEN AND TO HATCHABILITY

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The presence of vitamin E in eggs was established early in the investigations on the occurrence of this factor. Evans and Burr ('27) describe rat feeding experiments which showed that one-third of a whole egg fed daily as a supplement to a vitamin E-deficient diet permitted normal reproduction. However, they failed to obtain litters when 100 mg. of an ether extract of egg yolk when fed daily as a curative dose to female rats suffering from vitamin E-deficiency. Card et al. ('30) have recently studied the performance of pullets raised on a vitamin E-deficient diet. Their experiments indicated that vitamin E was essential to normal reproduction in the fowl. In studying the possible relationships of vitamin E to egg hatchability and the development of the chick, Adamstone ('31) showed that fertile eggs produced by pullets fed on vitamin E-deficient rations failed to hatch a normal proportion of healthy chicks. The embryos from eggs produced on the deficient diets usually died by the end of the fourth day of incubation or shortly thereafter. In discussing embryonic mortality and its probable cause in the chick, Byerly ('30) showed that the early peak in the mortality curve occurred on the third day, and also reported the frequent occurrence of a peak in the curve at about the middle of the incubation period.

The possible destruction or inactivation of vitamin E in the diet is suggested from the work of Mattill ('27), who reported results showing the high sensitivity of vitamin E to the destructive action of rancidity in fats. That the efficacy of a given source of vitamin E depends upon the presence or absence of certain associated fats which function either as pro- or anti-oxidants was further shown by Cummings and Mattill ('31), who attributed the inactivation of the vitamin to the action of peroxides developed during auto-oxidation of fats.

The results presented in this paper on the influence of the diet of the hen on the vitamin E content of the egg were obtained during the progress of studies on the nutritive aspects of egg composition which have been in progress for a number of years. Work has already been reported on some relationships of the diet of the hen to the chemical composition (Titus et al., '33), the hatchability (Byerly et al., '33 a, b), and the vitamins A, B and G contents (Ellis et al., '33) of the eggs. Diets low in vitamin E or especially supplemented in this factor were also fed. Determination of the differences in vitamin E content of eggs produced on different diets as well as studies on the presence and stability of vitamin E in the hen diets were made by means of rat feeding tests.[1]

PLAN OF EXPERIMENT

The vitamin E studies extended over a 3-year period on eggs obtained from three series of poultry feeding experiments run in successive years from 1930 to 1933. Each poultry feeding experiment was started in September and was terminated the following August. Rhode Island Red pullets or

[1] The experimental feeding of the hens was conducted under the direction of Harry W. Titus, while the hatchability studies were made under the supervision of Theodore C. Byerly, of the Office of Poultry Investigations. The investigations on vitamin E described in this paper were carried out in the nutrition laboratory under the supervision of N. R. Ellis, also of this division. The material presented in this paper was taken from the dissertation presented to the School of Hygiene and Public Health of the Johns Hopkins University by the author in partial fulfillment of the requirements for the degree of doctor of science. The interest and advice of Prof. E. V. McCollum in the problem is gratefully acknowledged.

yearling hens were used in all the work. The housing and management together with the general plans for compounding the diets of the hens have been described in earlier papers by Titus et al. ('33), Byerly et al. ('33) and Ellis et al. ('33).

Most of the vitamin E studies have been related to comparative work between a normal hen diet on the one hand and various modifications of a deficient diet on the other. The normal diet (no. 1) consisted of 72.3 per cent of the basal mixture of yellow corn meal, wheat bran, rolled oats and alfalfa leaf meal, 20 per cent of a protein supplement composed of meat meal, fish meal and dried buttermilk, together with minerals and cod liver oil. The five deficient diets studied contained pearled hominy, minerals and cod liver oil, together with protein and vitamin supplements. The protein level was kept at 20 per cent by adjustment of the hominy and meat scrap or buttermilk. Following is the list of diets showing the protein and vitamin supplements:

No. 2 Dry yeast cells (15 per cent) and meat scrap.
No. 3 Rice bran (15 per cent) and meat scrap.
No. 3a The no. 3 diet treated with ferric sulphate to inactivate the vitamin E.
No. 4 Dried buttermilk.
No. 5 Wheat germ (15 per cent) and meat scrap.
No. 6 Meat scrap (the basal deficient diet) and one head of lettuce daily for every seven birds.

Diets 1 and 3 were used all 3 years and the other diets during a single year. The eggs produced by the birds on these diets were studied both for their hatchability[2] and their vitamin E content. The testing of the eggs produced, as well as the diets fed to the hens for their vitamin E content were carried out on rats according to the general technic used by Evans and Burr ('27) and further studied on male rats by Mason ('29).

Male and female rats, when 4 weeks old, were placed in individual cages, with raised screen bottoms, and fed a basal vitamin E-deficient diet. This diet consisted of: alcohol ex-

[2] The results of hatchability studies made under the supervision of Dr. Theodore C. Byerly were kindly made available for reference in the present paper in order to show certain relationships of embryonic mortality to the vitamin E content.

tracted casein 20 per cent; lard 15 per cent; minerals[3] 4 per cent, agar 1 per cent; dry yeast 8 per cent; and corn dextrin 52 per cent. The cod liver oil was fed in separate glass containers to prevent its coming in contact with the lard in the diet.

The young females were mated for initial pregnancies at the age of 100 days. After observance of the mating plug, the animals were left in individual cages, examined for the implantation sign at the appropriate time, then weighed daily until delivery of litter or if no litter resulted until approximately the twenty-third day. Evidence of resorption rested primarily upon the weight curve. After initial resorption, feeding of the test material was started and the females again mated.

The testing of the poultry diets was done by supplementing the vitamin E-deficient rat diet with daily feedings at various levels of the poultry diet or of the substitution of the poultry diet for the vitamin E-deficient diet. Since rancidity has been shown to be an important factor in the destruction of vitamin E, the poultry diet supplements were fed in separate containers in order to prevent their coming in contact with the basal rat diet.

The eggs for the reproductive studies were collected and stored at approximately 7°C. The eggs were seldom over 2 weeks old when used, and were prepared for feeding by mixing the entire egg and pipetting the desired amount into small dishes. The egg testing was done during the spring and summer months during the period of maximum egg production. Lettuce and wheat germ were also tested both from the point of view of a check on the performance of the rats and on the material used in the hen diets. In the usual case the test material was fed approximately 2 weeks prior to conception and throughout the gestation period. All feedings

[3] Calcium carbonate 75.1 parts; calcium phosphate (dibasic) 107.6 parts, potassium phosphate (monobasic) 69.8 parts; potassium sulphate 22.0 parts; magnesium carbonate 21.0 parts; potassium carbonate 35.4 parts; sodium chloride 35.0 parts; ferric citrate 6.3 parts; potassium iodide 0.25 parts; manganese sulphate 1.0 parts; sodium fluoride 0.5 parts; potassium aluminum sulphate 0.5 parts; sodium silicate 1.0 parts; and copper sulphate 0.5 parts.

were strictly comparable even though carried over a longer period than is generally considered necessary for prevention of resorption. The sharpness of the results of the test feeding at definite daily levels attest to their validity. Emphasis was thus placed upon the comparative daily effective dose rather than the total amount of supplemental material. For example, eggs produced from hens on the normal diet fed at a 3-cc. level were only partially effective in preventing resorptions, while the 5-cc. level was totally effective. If a resorption as determined in the usual manner resulted in the second gestation, the female was used in test work again. After the female had undergone three or four resorptions, she was fed sufficient test material to produce a litter, thus proving her reproductive efficiency. After a litter, females were always allowed to mate and undergo resorption to insure depletion before being used for test work again.

Male rats were also used during the first year's work. Inasmuch as impairment in the male is permanent, the prophylactic type of experiment was used in order to study the preventive effect of the test material. In these tests the male rats were given daily test feedings of eggs or other material to be tested when started on the experiment. They were allowed to remain on this regimen for 119 days and were then studied for the dietary effects on testicular degeneration. In some cases functional tests were made, but, in general, the routine studies were made by; a) an examination of the spermatozoa by clipping the epididymis and noting motility, clumping and aspermia; b) histological study of both right and left testes; c) weight of trimmed testes. The weight of the testes was taken and the percentage of the normal was calculated according to Donaldson ('24).

RESULTS

Lettuce and wheat germ. One group of female rats was fed 5 gm. of fresh head lettuce daily during the entire gestation period. There were seven pregnancies in this series and four of these resulted in litters, while the remaining three termi-

nated in resorptions. Two controls fed lettuce supplements at the rate of 2.5 gm. daily in the prophylactic series produced normal and healthy litters. The lettuce fed in these experiments was the solid type of head lettuce obtained on the local market and no effort was made to select it according to the greenness of the leaves or to separate the leaves from the stock. Evidently this lettuce contained less vitamin E than that used by Evans and Burr ('27), since they found that 2.5 gm. daily doses of fresh green leaves fed therapeutically was effective in preventing resorptions.

Another control group was fed approximately 2.0 gm. of wheat germ daily. Eight pregnancies were studied in this series and each one resulted in a litter. Several rats in this series were allowed to re-mate a few weeks later, after being placed on the deficient ration without supplements of wheat germ or other sources of the reproductive factor. The resulting pregnancies also terminated in normal litters. In addition to the excess in the minimum effective dose indicated in feeding 2 gm. daily, further data are available showing that 1 gm. of wheat germ was sufficient to produce normal litters.

The poultry diets. The two diets most extensively used in the hatchability studies were the normal diet and the deficient rice bran diet. These diets were typical of those fed to pullets and hens which produced eggs of normal and of low hatchabilities. The diets were first tested for vitamin E content without the cod liver oil incorporated. As shown in table 1, little or no difference in their vitamin E content was found, since rats fed 5 gm. daily of either of the two diets were unable to produce litters, while 10 gm. of both diets permitted normal births.

The apparent difference in response of the rat and the hen to these two diets suggested a further examination of the diets with the cod liver oil incorporated into them at the same level and under comparable time and temperature conditions as fed to the hens. Accordingly, the cod liver oil was added at the rate of 2 per cent to the normal diet and the rice bran diet, which were then held in trays to permit free access of

air at a temperature of approximately 25°C. for 10 days. These rations were then tested for vitamin E as before. From table 1 it will be seen that the normal diet with cod liver oil added produced three resorptions and seven litters out of ten pregnancies studied. This result indicates that there was some destruction, although not as marked as in the rice bran

TABLE 1

The vitamin E content of poultry diets as determined by feeding tests with rats

DIET NO.	DAILY DOSE	NUMBER OF FEMALES	PREGNANCIES	RESORPTIONS	LITTERS
	gm.				
1	5	4	4	4	0
(Normal)	10	7	7	1	6
No cod liver oil)	ad lib.	7	7	1	6
3	5	4	4	4	0
(Rice bran)	10	6	6	0	6
No cod liver oil	ad lib.	13	13	1	12
1 (Normal) With cod liver oil	ad lib.	10	10	3	7
3 (Rice bran) With cod liver oil	ad lib.	7	7	6	1
1 (Normal) Iron treated	ad lib.	11	11	1	10
3a Iron treated rice bran diet	ad lib.	5	6	6	0

diet (no. 3) with cod liver oil added where six pregnancies resulted in resorptions and one in a litter. The rice bran oil present in this diet is suggested as a contributing factor in reducing the proportion of resorption cures.

Further study of the comparative destruction of the two diets was made by treatment with iron salts. The normal diet when so treated showed little loss in vitamin E potency.

The rice bran diet before treatment allowed the birth of normal litters in twelve of the thirteen pregnancies studied on ad libitum feeding, while after treatment resorptions (table 1) resulted in all of the six pregnancies studied.

The results of the tests on the poultry diets, including iron treatment as well as those with and without cod liver oil incorporated, apparently indicate that the rice bran possessed either a less amount of vitamin E or was more susceptible to destruction or inactivation of this factor than the normal diet.

The vitamin E content of the eggs. The eggs prepared as previously described were fed to vitamin E depleted female rats in doses of 3 cc., 5 cc., and 10 cc. daily. The eggs from the normal pen, when fed at 3 cc. levels (table 2) produced litters in five of the fourteen pregnancies during the 3 years' work. This level was apparently a borderline dose, since other rats fed at a level of 5 cc. produced litters in the ten pregnancies studied.

In contrast with the results on normal eggs, the rat tests on the eggs produced on the yeast diet yielded only one litter out of seven pregnancies on the 3-cc. and no litters out of seven pregnancies studied on the 5-cc. level. In the series on the eggs from the pens which received rice bran, little if any difference in vitamin E content was apparent between the diet treated with iron sulphate and the untreated diet. Eggs produced on diet no. 3 yielded no litters out of eight pregnancies on the 10-cc. level. Accordingly, the results were not unusual on diet no. 3a with thirteen resorptions out of fifteen pregnancies on the 5- and 10-cc. levels. In fact, the treatment of the hen diet with the iron salt appeared quite unnecessary after the findings on the no. 3 diet (rice bran).

The deficient eggs produced by the hens on the diet using buttermilk as the source of protein were studied on the 5-cc. daily dose only. Six pregnancies were observed and only one litter resulted. Three cubic centimeters of egg produced by hens fed the wheat germ diet were effective in producing four litters out of the five pregnancies studied as compared

to one litter in eight pregnancies with eggs from the pen with rice bran instead of wheat germ. The daily feeding of the 5 cc. of egg from the wheat germ diet resulted in litters in seven out of eight pregnancies as contrasted to two litters in seventeen pregnancies from the rice bran diet.

TABLE 2

The vitamin E content of whole eggs produced on different diets as shown by the curative effect on female rats

EGGS FROM DIET NO.	DAILY DOSE	NUMBER OF FEMALES	PREGNANCIES	RESORPTIONS	LITTERS
	cc.				
1	3	12	14	9	5
(Normal)	5	9	10	0	10
2	3	5	7	6	1
(Yeast)	5	5	7	7	0
3	3	6	10	8	2
(Rice bran)	5	14	18	15	3
	10	7	8	8	0
3a					
(Iron treated	5	7	7	7	0
rice bran diet)	10	7	8	6	2
4					
(Buttermilk)	5	5	6	5	1
5	3	5	6	2	4
(Wheat germ)	5	7	8	1	7
6	3	4	4	3	1
(Lettuce)	5	9	9	9	0
	10	7	7	6	1

The 3-, 5-, and 10-cc. levels of egg produced on the deficient diet supplemented with fresh lettuce were all ineffective in preventing resorptions in the female rats. Judging by the relative potency of lettuce and wheat germ as measured by the rat tests, the two hen diets were by no means comparable, since the diet with wheat germ was considerably more potent than that with lettuce.

The prophylactic tests with male rats. Studies with approximately seventy male rats on the potency of the eggs produced on the deficient diets supplemented with yeast and with rice bran, as well as those from the normal pen were made. The eggs from the normal pen were shown to be definitely more potent in their vitamin E content than those

TABLE 3

The hatchability and first 4-day mortality of the eggs produced on the diets used in the vitamin E studies

EGGS FROM DIET NO.	NUMBER OF EGGS USED	MORTALITY IN FIRST 4 DAYS OF INCUBATION	HATCHABILITY
		per cent	*per cent*
1 (Normal)	2237	7.0	73.0
2 (Yeast)	734	29.0	16.0
3 (Rice bran)	1836	25.0	26.0
3a (Iron treated rice bran)	168	42.0	18.0
4 (Buttermilk	887	22.0	27.0
5 (Wheat germ)	305	8.0	46.0
6 (Lettuce)	144	17.0	22.0

from the deficient pens. The results from the male tests support, in general, the female rat tests and the hatchability results on the eggs from the respective pens. One cubic centimeter of egg from hens on the normal diet was protective against marked degenerative changes, while 1 cc. of egg from hens on the deficient diets was not generally protective. However, protective feeding tests with male rats and their wide variation in degenerative changes as shown by weight of

the testes and histological section make tests with male rats less reliable and hence not as accurate a measure as the female series.

The hatchability of the eggs. Table 3 gives the approximate average hatchabilities of fertile eggs taken from the same pens as used in the vitamin E studies. A high mortality was noted in the first 4 days of the incubation period in the eggs from the yeast, rice bran and buttermilk diets, while the wheat germ showed an early mortality little different from the normal diet. The lettuce diet appeared somewhat less effective than the wheat germ, but more effective than any of the other deficient diets in the prevention of the early embryonic mortality.

Only the normal diet produced eggs of a reasonably high hatchability. All the other diets studied showed low hatching percentages ranging from 16 per cent on the yeast to 46 per cent on the wheat germ diet which indicated that a deficiency in some factor or factors persisted in spite of the various supplements added to or substituted in the deficient mixture of hominy and meat meal. The evident relation of vitamin E to the first 4-day mortality of the embryos is indicative of the important role vitamin E undoubtedly played in reducing the number of chickens hatched.

DISCUSSION

It is evident from the data presented that the vitamin E content of eggs may vary through a considerable range. However, the diet of the hen was the determining factor in producing the differences. While eggs produced on a normal diet were partially effective at a 3-cc. and fully effective at a 5-cc. daily level in the curative type of experimental rat test, 10 cc. of egg from deficient diets were insufficient. Thus, the eggs from the normal diet were fully three times as potent in vitamin E as those from deficient diets. In numerous instances, after a female had undergone a resorption on a given level of deficient eggs, she was again placed on the same level of egg from the same pen during the next pregnancy.

Most of these gestations also resulted in resorptions, while other cases on normal eggs showed cures in the first period of feeding. Frequent pregnancy histories of this nature make the contrast in results all the more striking.

The eggs from the normal and the wheat germ diets showed the highest vitamin E content along with the lowest embryonic mortality during the first 4 days of incubation. The per cent hatchability also showed the eggs from these two diets to rank first and second, respectively. However, the rather wide difference in actual values for hatchability as compared to the agreement in results on vitamin E content shown by the rat tests indicate that a factor or factors other than vitamin E were still lacking in the wheat germ diet (no. 5). Further study of the relative ranking of the eggs shows that the yeast (no. 2) and the treated rice bran diets were the poorest in vitamin E and in hatchability. The eggs produced on the rice bran diet (no. 3) appeared to contain little, if any, more vitamin E, although the hatchability was somewhat better than for the iron treated diet. Before incorporation of cod liver oil, the rice bran diet possessed a moderate amount of vitamin E comparable to that of the normal diet. However, rat tests on the complete diet (no. 3) as fed to the hens substantiate the findings on the low vitamin E content and low hatchability of the eggs produced. The inequality between lettuce and wheat germ as it affected the eggs was undoubtedly a question of relative amounts consumed by the hens. In other words, the potency of the head lettuce used was lower than estimated in formulating the diets and the amount supplied did not meet the minimum requirements of the birds. Buttermilk in the diet (no. 4) appeared to be less effective in prevention of the first 4-day mortality, but more effective in improving the hatchability than lettuce. In general, however, the hatchability, the freedom from embryonic mortality early in incubation and the vitamin E content of the eggs rank in approximately the same order.

In studying dietary deficiencies such as these, other possible variables such as vitamins A, B, D and G, as well as

possible protein deficiencies and mineral balance have not been overlooked. The fat soluble vitamins A and D were amply supplied in the diets and the experiments of Ellis et al. ('33) have shown adequate vitamin A content of eggs from hens receiving the normal and rice bran diets. These authors further showed that variations in the vitamin B content of the eggs may be produced by diets such as those used in the present studies, but such differences were not directly related to the hatchability of the eggs. The constitution of the diets used in the present studies, however, do not indicate the possibility of vitamin B deficiency. Further experiments of Ellis et al. ('33) do not show pronounced decrease in the vitamin G content of eggs produced on diets very deficient in this factor as compared to the normal diet. The possibility of mineral balance can be ruled out, because the mineral content of all the diets including the normal diet was essentially the same.

The quality of protein in the diets has been suggested by Byerly et al. ('33) as a factor in hatchability in connection with the present studies. However, the use of milk protein in the buttermilk diet effected little change from that of the meat protein in the rat.

From the hatchability standpoint, then, the richness of the diet in vitamin E and the ability of the hen to transmit this factor in an active form into the egg in sufficient amounts becomes a matter of considerable importance. It is true that a somewhat unusual dietary regimen was necessary to produce the differences observed in the present experiments. The requirements of the hen in relation to the supply in ordinary feeds does not appear to be excessive, yet certain combinations of feeds in one way or another may result in the involvement of vitamin E in hatchability. That it is an essential constituent of the diet of the hen appears reasonably certain.

SUMMARY AND CONCLUSIONS

Studies were conducted on the vitamin E content of eggs with relation to the diet of the hen and the hatchabilities of the eggs. By varying the diets in different pens it was found possible to produce eggs varying widely in their hatchabilities. The normal diet was partially effective at a 3-cc. level of eggs and a 5-cc. level was fully effective in curing vitamin E-deficiency in female rats. The eggs from those deficient diets which showed low hatchability were ineffective even at a 10-cc. level. The prophylactic egg tests with male rats yielded results which agreed, in general, with the female rat tests.

The normal diet which produced eggs of moderately high hatchability was found to be potent in vitamin E when tested with female rats. The diets which produced eggs of low hatchability particularly marked by a high first week embryonic mortality were found to be low or lacking in vitamin E, providing cod liver oil was incorporated in the diet of the rats. In the absence of the latter, the rat tests showed little difference in vitamin E content from the normal diet.

When the deficient diet which produced eggs lacking in vitamin E and low in hatchability was supplemented with 15 per cent of wheat germ, the eggs were comparable in vitamin E content to eggs produced on the normal diet. On the other hand, one head of lettuce per seven birds daily did not increase the vitamin E content or markedly improve the hatchability, although it reduced the first 4-day mortality over that of the deficient diet.

It is concluded from the data obtained that vitamin E may be a limiting factor in the hatchability of eggs on certain types of diets and that one manifestation of vitamin E-deficiency in the chick is the first-week embryonic mortality. The deficiency can be greatly reduced by supplying vitamin E in the poultry diet.

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LEUKOPENIA AND ANEMIA IN THE MONKEY RESULTING FROM VITAMIN DEFICIENCY [1]

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The discovery that cataract may be produced in rodents by restricting them to a diet deficient in vitamin G (Day, Langston and O'Brien, '31; O'Brien, '32; Langston and Day, '33, '34; Langston, Day and Cosgrove, '33; Yudkin, '33; Day and Langston, '34) led us to attempt similar experiments with monkeys. Before advanced cataractous changes developed, however, the monkeys died of a fulminating blood disease, characterized by marked anemia, leukopenia, gingivitis, and diarrhea. It is the purpose of this paper to report briefly the production of this hitherto unrecognized deficiency syndrome.

EXPERIMENTAL

The young rhesus monkeys (Macaca mulatta) used in these experiments were purchased from animal dealers. At the time they were placed on the experimental diet they weighed between 2.0 and 2.7 kg. each, and although histories were not available, from their weights and condition of dentition (Hartman and Straus, '33) they appeared to be about 2 years old. After a period of conditioning and observation, they were given a diet similar to that used in the production of

[1] The expenses of this investigation were defrayed in part by a grant from the Committee on Scientific Research of the American Medical Association.

cataract in rats. The daily ration for each monkey was as follows:

	Grams
Casein, Sherman and Spohn ('23)	10
Whole wheat, ground	15
Polished rice	30
Cod liver oil[3]	3
Salt mixture, Osborne and Mendel ('19)	4
Sodium chloride	2
Orange	4

The rice, casein, salt mixture and salt were cooked with sufficient water in a double boiler for 1½ hours, the wheat was then added and the cooking continued for another ½ hour. The cod liver oil was mixed with the ration just before feeding it. The orange was fed separately.

This diet is believed to be adequate, qualitatively and quantitatively, with respect to protein, inorganic elements, fatty acid, vitamins A, B(B_1), C, and D, but is deficient in vitamin G(B_2) and possibly some of the less well-known vitamins. Since Harden and Zilva ('20) found that 2 cc. of orange juice daily protected monkeys from scurvy, our animals received an adequate amount of ascorbic acid, yet the 4 gm. of orange given them supplied less than one Bourquin-Sherman unit of vitamin G (Day and Darby, '33). We are not convinced that the blood picture in the monkey here reported was the result of deprivation of the rat-growth vitamin G. It does appear probable, however, that the symptoms which developed were the result of a lack of some vitamin or vitamins.

The animals were observed daily, and at intervals of 1 week, or oftener, were taken from their cages and bled from the ear for hematological and chemical analyses. Total leukocyte and erythrocyte counts were made in duplicate, and smears for differential counts were made in the usual manner and stained with Wright's stain. Hemoglobin was determined by the acid hematin method of Newcomer, using a standard glass color disc for the colorimetric comparisons. Blood glucose was determined by the micro method of Folin ('28), and

[3] Supplied by the E. L. Patch Company, Boston.

cholesterol by the Leiboff method ('30). Urea was estimated by the micro method of Keller (Kolmer and Boerner, '31). The animals were given careful physical examinations, including observations of the mouth. Ophthalmoscopic examinations were frequently made. After the onset of the hypocythemia, blood determinations were made at semiweekly or daily intervals. At death autopsies were performed, and tissues were taken for histological examination. The pathological findings, including eye and bone marrow changes, will be reported later.

RESULTS AND DISCUSSION

In general, the monkeys ate the diet well, and although all showed a loss of appetite during the final stages of the disease, we feel that the blood changes were not due to anorexia, since the anemia and leukopenia preceded the loss of appetite. The animals on the deficient diet all lost weight, and showed marked emaciation before death. With one exception, the monkeys all developed a diarrhea within a short time after being placed on the diet. In some cases the diarrhea was intermittent, and in others continuous, with yellow or greenish, watery stools. We experienced some trouble with dysentery in our animals—a condition not uncommonly encountered by investigators working with imported rhesus monkeys. Since the deficiency appeared to predispose to an attack of a latent dysentery, stool cultures were made at frequent intervals, and animals showing positive tests for pathogenic organisms were excluded from the series.[3] Such animals usually died of an acute attack of dysentery, with large amounts of blood and mucus in the stools. The diarrhea caused by the deficiency, uncomplicated with dysenteric infections, was not characterized by blood and mucus, however, and did not appear to produce any marked ill effects upon the monkeys.

[3] A number of animals that died with clinical symptoms of dysentery yielded positive stool cultures for Shigella paradysenteriae, Hiss variety. One yielded a positive culture for Sonne variety.

In a series of seven monkeys dying of the deficiency, uncomplicated by any known dysentery or other infections, hematological data were obtained upon six of the animals, and blood chemical analyses were made upon five. Although there was some variation between individuals, the disease ran a rather typical course in all. As indicated in the introduction, the outstanding features were anemia and leukopenia, accompanied by, or followed by, an ulcerative gingivitis. The hematological and blood chemical findings and oral lesions are discussed below under the appropriate headings.

Hemoglobin. Normal values for hemoglobin in the monkeys used in these experiments ranged from 10.0 to 14.0 gm. per 100 cc. of blood, although most normal values came within the somewhat narrower limits of 11.0 and 13.0 gm. Monkeys on the deficient diet, after a variable period in which the hemoglobin remained normal, showed a progressive and usually rapid decrease in pigment. The extent of diminution of hemoglobin depended somewhat upon the length of survival of the animal after the anemia developed. The average final content of hemoglobin was 7.10 gm., but one went as low as 2.27 gm. per 100 cc. of blood.

Erythrocyte count. Normal erythrocyte counts on the young monkeys were found to be between 4.5 and 6.0 million per cubic millimeter. In the experimental animals there occurred a decrease in red corpuscles coincident with and paralleling the decrease in hemoglobin. The count before death was less than 3.0 million cells in every case, and in one animal dropped to less than one million (955,000) erythrocytes per cubic millimeter of blood.

Leukocyte count. The normal range of white cells in young monkeys is rather high and quite variable in the experience of Wills and Bilimoria ('32), and this was found to be the case in our animals also. The monkeys on a mixed stock diet, and normal controls receiving the deficient diet plus yeast, had total white counts between 10,000 and 30,000. There was a marked difference between animals, and also a distinct variation from week to week in any one individual. Be-

cause of this wide variation and the lack of a definite normal standard, it is difficult to say just when a leukopenia developed in any given case. However, a definite drift downward usually started at about the same time as the decrease in hemoglobin and erythrocytes began. Minimum counts were below 5500 in every case; four monkeys out of six had white counts below 2000, and one animal had a final total leukocyte count of only 695 cells per cubic millimeter.

The differential counts were so variable that it is difficult to make any broad statement that holds true for all the animals. In general, however, as the leukopenia developed, the decrease in white cells appeared to be more at the expense of the neutrophils than of the lymphocytes. That is, there was frequently a relative neutropenia together with a relative lymphocytosis, although there was, of course, an actual lymphopenia as well as an actual neutropenia. One animal in its final count exhibited 94 per cent lymphocytes and 4 per cent neutrophils. The differential counts will be given in detail in a later report.

Blood cholesterol. Monkeys on a normal stock diet (grains, sunflower seeds, bread, fruits and vegetables) had cholesterol values as high as 200 mg. per 100 cc. of blood, but a normal control receiving the deficient diet supplemented with yeast showed cholesterol as low as 130 mg. Cholesterol in the blood of animals on the deficient diet fell progressively, decreasing to 100 mg. or less in all five of the animals on which determinations were made, and in one animal going as low as 62 mg. per 100 cc. of blood.

Blood glucose. The animals were found to have blood sugar levels between 100 and 140 mg. per 100 cc., although excitement or exercise resulted in glucose levels higher than 140 mg. The experimental animals had normal blood sugar values until the last day or two of life, when there was a decrease below normal, going as low as 60 mg. before death. The blood samples were always drawn before the daily feeding, and carbohydrate tolerance tests were not made.

Blood urea. The normal values were found to be between 12 and 24 mg. urea N per 100 cc. blood. There was no signifi-

cant variation from normal in the experimental animals until the last 2 days of life, at which time there was an increase. Blood samples taken from the moribund animals were found to contain between 40 and 47 mg. urea N per 100 cc. This terminal increase possibly is not important, as it may have been due to failing circulation. Sufficient blood was obtained from the heart of two animals at death to make determinations of creatinine possible. Each had less than 2.0 mg. creatinine per 100 cc. of blood, so it may be concluded that there was no retention of this substance.

Oral lesions. The only consistent physical finding was an ulceration of the margins of the gums. This lesion usually developed within a short time after the onset of the leukopenia, first appearing as a slight recession of the gums, and later as a distinct yellowish ulceration. Upon wiping off the necrotic material with a cotton swab, mild bleeding resulted. This ulceration usually developed along the incisors first, and in several animals caused the teeth to fall out. In one monkey the ulcerated region was chiefly along the canine and molars, and progressed until a large area of bone was exposed. The gums were pale and there were no marked spontaneous hemorrhages. No other oral lesions were observed.

With the exception of a single, small, perforating ulcer on the paw of one animal, no skin changes were seen.

The survival period on the deficient diet varied from 26 to 81 days, with an average of 56 days. Since a normal control receiving the deficient diet plus dried brewers' yeast has lived more than 200 days without showing any of the signs discussed above, it appears that this progressive hypocythemia was due to a deficiency of some substance which is found in yeast. It seems probable that the syndrome was due to a lack of some component of the vitamin B complex.

Since black tongue in dogs may be produced by feeding a diet similar to the diet we used, it would have been natural to expect an analogous condition in the monkey. However, because of the nature of the oral lesions and the absence of dermatitis, it is apparent that the condition in the monkey

herein reported does not clearly resemble either black tongue or pellagra.

SUMMARY

Young monkeys (Macaca mulatta) were given a diet believed to be adequate with respect to protein, inorganic elements, fatty acid, vitamins A, B(B_1), C, and D, but deficient in vitamin G(B_2) and possibly deficient in other less well-known organic substances which may be essential to the nutrition of the monkey. After a varying period on the diet, the animals developed a fulminating, fatal blood disease, characterized by anemia and leukopenia. Ulceration of the gums was a consistent accompaniment of the hypocythemia, and diarrhea was common. There was a progressive decrease in blood cholesterol, but no significant change in the blood sugar level. Blood urea remained normal until the last 2 days of life, at which time the level of this substance became somewhat elevated. There was no retention of creatinine.

The deficient diet supplemented with dried brewers' yeast supported good growth and prevented the deficiency syndrome.

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